Editors:

ECI2018 Congress President
Prof. Dr. Marieke van Ham
Head Dept. of Immunopathology
Sanquin Research and SILS, Faculty of Science, University of Amsterdam
Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands
m.vanham@sanquin.nl

ECI2018 Scientific Program Committee President
Prof Dr. Jaques Neefjes
Leiden University Medical CenterDepartment of Cell & Chemical Biology
P.O. Box 9600, 2300 RC Leiden, The Netherlands
j.j.c.neefjes@lumc.nl

ECI2018 Local Organizing Committee President
Janneke N. Samsom, PhD
Erasmus University Medical Center
Laboratory of Pediatric Gastroenterology
P.O. Box 2040, 3000 CA Rotterdam, The Netherlands
j.samsom@erasmusmc.nl

ECI2018 and EFIS President’s Office
Christina Helbig, PhD
Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands
helbig@efis.org

Graphic Design: Christina Helbig

Coordination:

Wiener Medizinische Akademie GmbH
Alser Strasse 4, 1090 Vienna, Austria
+43 1 405 13 83 30
eci2018@medacad.org
www.eci2018.org

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GUIDELINES TO READ THE ABSTRACT BOOK

The scientific program is divided into 5 main topic tracks:

Track A: Immune development and differentiation
Track B: Tumor immunology and therapy
Track C: Autoimmunity, allergy and transplantation
Track D: Infections and microbial immune regulation
Track E: Immunomics – Technical advances and big data

There are 10 types of sessions generated from invited and submitted abstracts:

Keynote Lectures (KL): oral presentations by invited keynote speakers
Symposia (S): oral presentations by invited speakers
Joint Symposium (JS): oral presentations by invited speakers
Educational Sessions (EDU): oral presentations by invited speakers
Men and Women in Immunology (MWI): oral presentations by invited speakers
EFIS President’s Symposium (EP): oral presentations by invited speakers
Bright Sparks Workshop (BS): oral presentations from selected abstracts
Workshop (WS): oral presentations from selected abstracts
Late Breaking Hot Topics (HT): oral presentations from selected abstracts
Guided Poster Session (P): poster presentations from selected abstracts

How to read the presentation numbers – for example: WS.B1.06.04

**WS.B1.06.04:**  
WS stands for one of the following session types

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>KL</td>
<td>Keynote Lecture</td>
</tr>
<tr>
<td>S</td>
<td>Symposium</td>
</tr>
<tr>
<td>JS</td>
<td>Joint Symposium</td>
</tr>
<tr>
<td>EDU</td>
<td>Educational Session</td>
</tr>
<tr>
<td>MWI</td>
<td>Men and Women in Immunology</td>
</tr>
<tr>
<td>EP</td>
<td>EFIS President’s Symposium</td>
</tr>
<tr>
<td>BS</td>
<td>Bright Spark</td>
</tr>
<tr>
<td>WS</td>
<td>Workshop</td>
</tr>
<tr>
<td>HT</td>
<td>Late Breaking Hot Topic</td>
</tr>
<tr>
<td>P</td>
<td>Poster Session</td>
</tr>
</tbody>
</table>

**WS.B1.06.04:**  
B stands for one of the following tracks:

<table>
<thead>
<tr>
<th>Track</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Immune development and differentiation</td>
</tr>
<tr>
<td>B</td>
<td>Tumor immunology and therapy</td>
</tr>
<tr>
<td>C</td>
<td>Autoimmunity, Allergy and Transplantation</td>
</tr>
<tr>
<td>D</td>
<td>Infections and microbial immune regulation</td>
</tr>
<tr>
<td>E</td>
<td>Immunomics - Technical advances and big data</td>
</tr>
</tbody>
</table>

**WS.B1.06.04:**  
1 indicates the subtopic within the respective track

**WS.B1.06.04:**  
06 indicates the chronological order of sessions within the respective subtopic

**WS.B1.06.04:**  
04 indicates the chronological order of presentations within the respective session

Thus, WS.B1.04.04 indicates the fourth talk in workshop 04 of subtopic B1!

ANNOTATIONS

In the following we are publishing the abstracts as submitted by the authors.
Missing session numbers represent sessions with no abstracts associated. Missing presentation numbers represent withdrawn or embargoed abstracts which have not been received as per date of publication.
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# TABLE OF CONTENTS

## KEYNOTE LECTURES
- KL.01 Serendipities of acquired immunity ............................................. 11
  - KL.02 Trained immunity: reprogramming innate immunity to protect against infections 12
  - KL.07 Microbiota and cancer therapeutics ........................................... 12
  - KL.08 The maintenance and mobilization of resident immune memory cells 12

## SYMPOSIA
- S.A2 Immune development and aging from the cradle to the grave .................. 13
- S.A3 Immunomonitoring and biomarkers ................................................. 14
- S.A4 Germinal centers and B cell differentiation ........................................ 14
- S.A5 Initiation of immune responses ......................................................... 15
- S.B1 Tumor vaccination principles and immuno therapy .............................. 15
- S.B2 Environmental regulation anti-tumor responses ..................................... 15
- S.B3 The Yin and Yang of T-cell regulation .................................................. 16
- S.B4 T cell activation and exhaustion ......................................................... 16
- S.C1 Maintenance and local regulation of tissue specific immunity ................. 16
- S.C2 Immune signalling and therapy in autoimmunity ..................................... 16
- S.C3 Transplantation ..................................................................................... 17
- S.C4 Manipulation of tolerance ..................................................................... 17
- S.C5 Allergy, asthma and therapy ............................................................... 18
- S.C6 Innate control of inflammation and tissue repair..................................... 18
- S.D2 Innate lymphoid cells - a topic of debate ............................................. 19
- S.D3 Novel approaches to vaccinology ......................................................... 19
- S.D4 Exploiting host pathogen interaction .................................................... 20
- S.E1 Visualizing immune responses ............................................................ 20
- S.E2 How to handle big data and can we do this? ......................................... 20
- S.E4 Cell communication and signaling in the immune system ....................... 21

## JOINT SYMPOSIA
- JS.01 Trends in Vaccinology ........................................................................ 22
- JS.02 Systems Immunology for stratifying patients with autoimmune diseases 23
- JS.03 Antigen Presentation in Health and Disease ......................................... 23
- JS.04 HLA in Transplantation and Autoimmunity ........................................ 24
- JS.05 Combinatorial approaches to develop targeted immunotherapeutics ...... 24
- JS.06 Subversion of phagocytosis in innate immunity: from efferocytosis to pathogen interaction ................................................................. 24
- JS.07 Cytometry building bridges ............................................................... 25
- JS.08 The gut microbiota and the IgA antibody production in health and disease 25
- JS.09 Innate host pathogen interactions ...................................................... 25
- JS.10 Innate host pathogen interactions ...................................................... 25

## EDUCATIONAL SESSIONS
- EDU.01 Systems Biology for Immunology: Help with the Complexity ............ 26
- EDU.02 The Utility of Theories in Immunology ............................................ 27
- EDU.03 Immunology of extracellular vesicles ................................................ 27

## EFIS PRESIDENT'S SYMPOSIUM
- EP.01 T Cell immunity in the front line ...................................................... 29

## LATE BREAKING HOT TOPICS
- HT.04 Late Breaking Hot Topic 4 .................................................................. 31
- HT.05 Late Breaking Hot Topic 5 .................................................................. 32
- HT.06 Late Breaking Hot Topic 6 .................................................................. 32
# TABLE OF CONTENTS

**BRIGHT SPARKS WORKSHOPS** ................................................................. 33

BS.A.01 Bright Sparks A ................................................................. 34
BS.B.01 Bright Sparks B ................................................................. 35
BS.C.01 Bright Sparks C ................................................................. 36
BS.D.01 Bright Sparks D ................................................................. 37

**WORKSHOPS** ................................................................................... 39

WS.A1.01 Myeloid lineage specifications ........................................... 40
WS.A2.01 T cells in aging ................................................................. 41
WS.A2.02 Immune development and neonatal responses .................. 42
WS.A2.03 Immune cell aging and differentiation .............................. 43
WS.A2.04 Evolution of immune responses in health and disease ....... 44
WS.A3.01 Immunobiomarkers in autoimmunity and beyond .......... 45
WS.A3.02 Biomarkers of adaptive immunity .................................... 47
WS.A3.03 Immune markers in malignancies .................................... 48
WS.A4.01 Germinal center reactions ............................................... 49
WS.A4.02 Regulation of B cell development and differentiation ...... 50
WS.A5.01 Innate effectors in the onset of immune responses ........... 51
WS.A5.02 Early T cell functions in immune responses .................... 53
WS.A5.03 DC and tissue-derived cellular responses .......................... 54
WS.A6.01 Lessons learned from genetic defects .............................. 55
WS.B1.01 Immune checkpoints in anti-tumor therapy ..................... 56
WS.B1.02 Novel targets in anti-cancer immune therapy ................... 57
WS.B1.03 Genetically engineered TCR for immunotherapy .......... 59
WS.B1.04 Antigen specificity in anti-tumor immunity ...................... 60
WS.B1.05 Anti-tumor immunology .................................................. 61
WS.B1.06 Anti-tumor strategies ....................................................... 62
WS.B2.01 Environmental regulation of anti-tumor responses .......... 63
WS.B2.02 Tumor immune surveillance and evasion ....................... 65
WS.B2.03 Innate anti-tumor immunity ............................................ 66
WS.B3.01 Molecular regulation of T cell responses ........................ 67
WS.B3.02 T cell mediated immune regulation in tumors ............... 68
WS.B3.03 T cell responses in health and disease ............................. 69
WS.B4.01 Molecular control of T cell activation and exhaustion ...... 70
WS.B4.02 Targeting checkpoints ..................................................... 72
WS.C1.01 Regulation in tissue specific autoimmunity 1 .................... 73
WS.C1.02 Immune regulation at mucosal sites ................................. 74
WS.C1.03 Cytokine and transcription factor mediated immune regulation .... 75
WS.C1.04 Regulation in tissue specific autoimmunity 2 .................... 76
WS.C2.01 Signaling in autoimmunity .............................................. 78
WS.C2.02 Neuroinflammatory disorders ......................................... 79
WS.C2.03 Pathophysiology of autoimmune diseases .................... 80
WS.C2.04 Therapy of autoimmune disorders .................................. 81
WS.C3.01 Transplantation - pathogenesis and early diagnosis ......... 83
WS.C3.02 T regulatory cells derived and other cellular therapies in transplantation .... 84
WS.C4.01 Manipulation of tolerogenic pathways ........................... 85
WS.C4.02 Manipulation of Tolerance by FoxP3+ T Regs ................... 87
WS.C5.01 Physiopathology of allergic disorders ............................. 88
WS.C5.02 Immunotherapy of allergic disorders ............................. 89
WS.C6.01 Acquired immunity crosstalk with inflammation .......... 90
# TABLE OF CONTENTS

| WS.C6.02 | New mediators in inflammation and its resolution | .................................................. | 91 |
| WS.C6.03 | Immune cells in tissue fibrosis | .......................................................... | 92 |
| WS.D1.01 | Mucosal immune regulation | .......................................................... | 94 |
| WS.D1.02 | Innate responses and immune signaling | .................................................. | 95 |
| WS.D1.03 | Regulation of effector immune responses | .................................................. | 96 |
| WS.D2.01 | Molecular properties of innate immune cells | .................................................. | 97 |
| WS.D2.02 | Molecular and cellular features of ILCs | .................................................. | 98 |
| WS.D3.01 | Novel vaccine approaches to intracellular pathogens | .......................................... | 99 |
| WS.D3.02 | Novel vaccine approaches for viruses | .................................................. | 100 |
| WS.D4.01 | Protective mechanisms for microbial pathogens | .................................................. | 102 |
| WS.D4.02 | Responses to mucosal microbial pathogens | .................................................. | 103 |
| WS.D4.03 | Immune sensing of microbial infections | .................................................. | 104 |
| WS.D4.04 | Virus-host interactions | .................................................. | 105 |
| WS.D4.05 | Innate-adaptive interface during microbial infections | .................................................. | 106 |
| WS.D4.06 | Bacterial infections and immune activation | .................................................. | 107 |
| WS.D4.07 | Innate immune responses and infection | .................................................. | 109 |
| WS.E1.01 | Visualizing immune responses | .................................................. | 110 |
| WS.E2E3.01 | Single cells to population dynamics and handling Big Data | .................................................. | 111 |
| WS.E4.01 | Cell communication and signaling in the immune system | .................................................. | 112 |

# POSTER PRESENTATIONS

| P.A1.01 | Myeloid lineage specification - Part 1 | .................................................. | 115 |
| P.A1.02 | Myeloid lineage specification - Part 2 | .................................................. | 119 |
| P.A2.01 | Immune development and aging from the cradle to the grave - Part 1 | .................................................. | 123 |
| P.A2.02 | Immune development and aging from the cradle to the grave - Part 2 | .................................................. | 128 |
| P.A2.03 | Immune development and aging from the cradle to the grave - Part 3 | .................................................. | 132 |
| P.A2.04 | Immune development and aging from the cradle to the grave - Part 4 | .................................................. | 137 |
| P.A3.01 | Immunomonitoring and biomarkers - Part 1 | .................................................. | 141 |
| P.A3.02 | Immunomonitoring and biomarkers - Part 2 | .................................................. | 145 |
| P.A3.03 | Immunomonitoring and biomarkers - Part 3 | .................................................. | 149 |
| P.A3.04 | Immunomonitoring and biomarkers - Part 4 | .................................................. | 153 |
| P.A3.05 | Immunomonitoring and biomarkers - Part 5 | .................................................. | 157 |
| P.A3.06 | Immunomonitoring and biomarkers - Part 6 | .................................................. | 161 |
| P.A3.07 | Immunomonitoring and biomarkers - Part 7 | .................................................. | 165 |
| P.A4.01 | Germinal centers and B cell differentiation - Part 1 | .................................................. | 168 |
| P.A4.02 | Germinal centers and B cell differentiation - Part 2 | .................................................. | 173 |
| P.A4.03 | Germinal centers and B cell differentiation - Part 3 | .................................................. | 177 |
| P.A5.01 | Initiation of immune responses - Part 1 | .................................................. | 180 |
| P.A5.02 | Initiation of immune responses - Part 2 | .................................................. | 184 |
| P.A5.03 | Initiation of immune responses - Part 3 | .................................................. | 188 |
| P.A5.04 | Initiation of immune responses - Part 4 | .................................................. | 192 |
| P.A5.05 | Initiation of immune responses - Part 5 | .................................................. | 195 |
| P.A5.06 | Initiation of immune responses - Part 6 | .................................................. | 198 |
| P.A5.07 | Initiation of immune responses - Part 7 | .................................................. | 201 |
| P.A6.01 | Lessons learned from the genetic defects - Part 1 | .................................................. | 205 |
| P.A6.02 | Lessons learned from the genetic defects - Part 2 | .................................................. | 208 |
| P.B1.01 | Tumor vaccination principles and Immunotherapy - Part 1 | .................................................. | 211 |
| P.B1.02 | Tumor vaccination principles and Immunotherapy - Part 2 | .................................................. | 215 |
| P.B1.03 | Tumor vaccination principles and Immunotherapy - Part 3 | .................................................. | 219 |
| P.B1.04 | Tumor vaccination principles and Immunotherapy - Part 4 | .................................................. | 223 |
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.B1.05 Tumor vaccination principles and Immunotherapy - Part 5</td>
<td>226</td>
</tr>
<tr>
<td>P.B1.06 Tumor vaccination principles and Immunotherapy - Part 6</td>
<td>230</td>
</tr>
<tr>
<td>P.B1.07 Tumor vaccination principles and Immunotherapy - Part 7</td>
<td>234</td>
</tr>
<tr>
<td>P.B1.08 Tumor vaccination principles and Immunotherapy - Part 8</td>
<td>238</td>
</tr>
<tr>
<td>P.B1.09 Tumor vaccination principles and Immunotherapy - Part 9</td>
<td>242</td>
</tr>
<tr>
<td>P.B2.01 Environmental regulation anti-tumor responses - Part 1</td>
<td>245</td>
</tr>
<tr>
<td>P.B2.02 Environmental regulation anti-tumor responses - Part 2</td>
<td>249</td>
</tr>
<tr>
<td>P.B2.03 Environmental regulation anti-tumor responses - Part 3</td>
<td>254</td>
</tr>
<tr>
<td>P.B2.04 Environmental regulation anti-tumor responses - Part 4</td>
<td>258</td>
</tr>
<tr>
<td>P.B2.05 Environmental regulation anti-tumor responses - Part 5</td>
<td>262</td>
</tr>
<tr>
<td>P.B2.06 Environmental regulation anti-tumor responses - Part 6</td>
<td>266</td>
</tr>
<tr>
<td>P.B2.07 Environmental regulation anti-tumor responses - Part 7</td>
<td>270</td>
</tr>
<tr>
<td>P.B3.01 T-cell regulation - Part 1</td>
<td>274</td>
</tr>
<tr>
<td>P.B3.02 T-cell regulation - Part 2</td>
<td>277</td>
</tr>
<tr>
<td>P.B3.03 T-cell regulation - Part 3</td>
<td>280</td>
</tr>
<tr>
<td>P.B3.04 T-cell regulation - Part 4</td>
<td>283</td>
</tr>
<tr>
<td>P.B4.01 T-cell activation and exhaustion - Part 1</td>
<td>287</td>
</tr>
<tr>
<td>P.B4.02 T-cell activation and exhaustion - Part 2</td>
<td>291</td>
</tr>
<tr>
<td>P.B4.03 T-cell activation and exhaustion - Part 3</td>
<td>294</td>
</tr>
<tr>
<td>P.C1.01 Maintenance and local regulation of tissue specific immunity - Part 1</td>
<td>298</td>
</tr>
<tr>
<td>P.C1.02 Maintenance and local regulation of tissue specific immunity - Part 2</td>
<td>302</td>
</tr>
<tr>
<td>P.C1.03 Maintenance and local regulation of tissue specific immunity - Part 3</td>
<td>306</td>
</tr>
<tr>
<td>P.C1.04 Maintenance and local regulation of tissue specific immunity - Part 4</td>
<td>310</td>
</tr>
<tr>
<td>P.C1.05 Maintenance and local regulation of tissue specific immunity - Part 5</td>
<td>314</td>
</tr>
<tr>
<td>P.C1.06 Maintenance and local regulation of tissue specific immunity - Part 6</td>
<td>317</td>
</tr>
<tr>
<td>P.C1.07 Maintenance and local regulation of tissue specific immunity - Part 7</td>
<td>321</td>
</tr>
<tr>
<td>P.C1.08 Maintenance and local regulation of tissue specific immunity - Part 8</td>
<td>324</td>
</tr>
<tr>
<td>P.C2.01 Immune signaling and therapy in autoimmunity - Part 1</td>
<td>328</td>
</tr>
<tr>
<td>P.C2.02 Immune signaling and therapy in autoimmunity - Part 2</td>
<td>332</td>
</tr>
<tr>
<td>P.C2.03 Immune signaling and therapy in autoimmunity - Part 3</td>
<td>335</td>
</tr>
<tr>
<td>P.C2.04 Immune signaling and therapy in autoimmunity - Part 4</td>
<td>339</td>
</tr>
<tr>
<td>P.C2.05 Immune signaling and therapy in autoimmunity - Part 5</td>
<td>342</td>
</tr>
<tr>
<td>P.C2.06 Immune signaling and therapy in autoimmunity - Part 6</td>
<td>346</td>
</tr>
<tr>
<td>P.C2.07 Immune signaling and therapy in autoimmunity - Part 7</td>
<td>350</td>
</tr>
<tr>
<td>P.C2.08 Immune signaling and therapy in autoimmunity - Part 8</td>
<td>353</td>
</tr>
<tr>
<td>P.C2.09 Immune signaling and therapy in autoimmunity - Part 9</td>
<td>357</td>
</tr>
<tr>
<td>P.C2.10 Immune signaling and therapy in autoimmunity - Part 10</td>
<td>361</td>
</tr>
<tr>
<td>P.C2.11 Immune signaling and therapy in autoimmunity - Part 11</td>
<td>364</td>
</tr>
<tr>
<td>P.C3.01 Bone Marrow Transplantation</td>
<td>367</td>
</tr>
<tr>
<td>P.C3.02 Regulatory Mechanisms in Transplantation</td>
<td>371</td>
</tr>
<tr>
<td>P.C3.03 Organ Transplantation, Genotyping</td>
<td>374</td>
</tr>
<tr>
<td>P.C3.04 MHC, Stem Cell Transplantation and Regulation</td>
<td>378</td>
</tr>
<tr>
<td>P.C4.01 Manipulation of tolerance - Part 1</td>
<td>382</td>
</tr>
<tr>
<td>P.C4.02 Manipulation of tolerance - Part 2</td>
<td>385</td>
</tr>
<tr>
<td>P.C4.03 Manipulation of tolerance - Part 3</td>
<td>389</td>
</tr>
<tr>
<td>P.C5.01 Allergy, asthma and therapy - Part 1</td>
<td>393</td>
</tr>
<tr>
<td>P.C5.02 Allergy, asthma and therapy - Part 2</td>
<td>397</td>
</tr>
<tr>
<td>P.C5.03 Allergy, asthma and therapy - Part 3</td>
<td>401</td>
</tr>
<tr>
<td>P.C5.04 Allergy, asthma and therapy - Part 4</td>
<td>406</td>
</tr>
<tr>
<td>P.C6.01 Innate control of inflammation and tissue repair - Part 1</td>
<td>408</td>
</tr>
</tbody>
</table>

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 9
TABLE OF CONTENTS

P.C6.02 Innate control of inflammation and tissue repair - Part 2 ................................................................. 411
P.C6.03 Innate control of inflammation and tissue repair - Part 3 ................................................................. 415
P.C6.04 Innate control of inflammation and tissue repair - Part 4 ................................................................. 418
P.C6.05 Innate control of inflammation and tissue repair - Part 5 ................................................................. 422
P.C6.06 Innate control of inflammation and tissue repair - Part 6 ................................................................. 426
P.D1.01 Microbiome, metabolites and the immune system - Part 1 ................................................................. 429
P.D1.02 Microbiome, metabolites and the immune system - Part 2 ................................................................. 434
P.D1.03 Microbiome, metabolites and the immune system - Part 3 ................................................................. 438
P.D1.04 Microbiome, metabolites and the immune system - Part 4 ................................................................. 442
P.D2.01 Innate Lymphoid Cells ........................................................................................................................ 446
P.D2.02 NK cells and innate immune mechanisms ............................................................................................ 450
P.D3.01 Novel approaches to vaccinology - Part 1 .......................................................................................... 453
P.D3.02 Novel approaches to vaccinology - Part 2 .......................................................................................... 457
P.D3.03 Novel approaches to vaccinology - Part 3 .......................................................................................... 461
P.D3.04 Novel approaches to vaccinology - Part 4 .......................................................................................... 465
P.D4.01 Exploiting host pathogen interaction - Part 1 ...................................................................................... 469
P.D4.02 Exploiting host pathogen interaction - Part 2 ...................................................................................... 473
P.D4.03 Exploiting host pathogen interaction - Part 3 ...................................................................................... 477
P.D4.04 Exploiting host pathogen interaction - Part 4 ...................................................................................... 481
P.D4.05 Exploiting host pathogen interaction - Part 5 ...................................................................................... 484
P.D4.06 Exploiting host pathogen interaction - Part 6 ...................................................................................... 488
P.D4.07 Exploiting host pathogen interaction - Part 7 ...................................................................................... 492
P.D4.08 Exploiting host pathogen interaction - Part 8 ...................................................................................... 495
P.D4.09 Exploiting host pathogen interaction - Part 9 ...................................................................................... 500
P.D4.10 Exploiting host pathogen interaction - Part 10 .................................................................................... 504
P.D4.11 Exploiting host pathogen interaction - Part 11 .................................................................................... 507
P.E1.01 Visualizing immune responses - Part 1 ............................................................................................... 511
P.E1.02 Visualizing immune responses - Part 2 ............................................................................................... 513
P.E2.01 How to handle big data? ....................................................................................................................... 516
P.E3E4.01 From single cells to population dynamics / Cell communication and signaling in the immune system ................................................................. 519
P.E4.01 Cell communication and signaling in the immune system ..................................................................... 522

AUTHOR INDEX .............................................................................................................................................. 527
KEYNOTE LECTURES
**KL01.1 Serendipities of acquired immunity**

**T. Honjo**
Kyoto University Institute for Advanced Study, Kyoto, Japan.

In 1992, we started working on PD-1 and found that this acts as a brake in the immune system. Then, in 2002, we discovered that PD-1 inhibition could be effective in treating cancer in animal models. After 22 years of study, this idea has borne fruit in a new, breakthrough immunotherapy that is being hailed as a ‘penicillin moment’ in cancer treatment. I believe that, just as a number of antibiotics developed in the wake of the discovery of penicillin now protect humans against threats of infectious diseases, this discovery will play a leading role in advancement of cancer immunotherapy so that in the future the fear of dying from cancer will cease to exist. Through evolution, vertebrate animals have developed immunity against infection by microorganisms. In the process, they incidentally acquired a sophisticated system for diversifying genomic information by combining gene fragments. It was doubly fortunate that the success in cancer treatment via PD-1 inhibition brought the realization that immunity, a “weapon” against infectious diseases, could also serve as a “shield” against cancer. It has been said that, whereas humankind’s greatest enemies in the 20th century were infectious diseases, cancer is the major foe in the 21st century. It is a pleasant surprise to discover that the acquired immunity system holds the keys to overcoming both of these difficult medical challenges.

**KL02.1 Trained immunity: reprogramming innate immunity to protect against infections**

**M. G. Netea**
Radboud University Medical Center, Nijmegen, Netherlands.

The inability of innate immunity to build an immunological memory, considered one of the main characteristics differentiating it from adaptive immunity, has been recently challenged by studies in plants, invertebrates, and mammals. Long-term reprogramming of innate immunity, that induces adaptive traits and has been termed trained immunity characterizes prototypical innate immune cells such as natural killer cells and monocytes, and provides protection against reinfeciton in a T-cell-independent manner. In contrast, reaction has been shown to be able to induce protection against reinfection in a lymphocyte-independent manner. Non-specific protective effects dependent on trained immunity have also been shown to be induced after BCG vaccination in humans. Specific signaling mechanisms including the dectin-1/Raf1 and NOD2-mediated pathways induce trained immunity, through induction of histone modifications (methylation, acetylation) and epigenetic reprogramming of monocyte function. Complex immunological and metabolic circuits link cell stimulation to a long-term epigenetic reprogramming of its function. The concept of trained immunity represents a paradigm change in immunity and its putative role in infection and inflammation may represent the next step in the design of future vaccines and immunotherapeutic approaches.

**KL07 Microbiota and cancer therapeutics**

**KL07.1 The unsuspected role of gut microbiota in cancer therapies**

**L. Zitvogel**
INSERM, Gustave Roussy Cancer Center, University Paris Saclay, Villejuif, France.

We recently highlighted the crucial role of gut microbiota in eliciting innate and adaptive immune responses beneficial for the host in the context of effective therapies against cancer (chemotherapies, immunotherapy based on immune checkpoint blockers).

1. **Context of cyclophosphamide (CTX):** Chemotherapeutic agents, by compromising, to some extent, the intestinal integrity, facilitate the gut permeability and selective translocation of Gram positive bacteria in secondary lymphoid organs. There, anti-commensal pathogenic Th17 T cell responses are primed, facilitating the accumulation of Th1 helper T cells in tumor beds post-chemotherapy as well as tumor regression. Importantly, the redox equilibrium of myeloid cells contained in the tumor microenvironment is also influenced by the intestinal microbiota, contributing to tumor responses. Hence, the anticancer efficacy of allylating agents is compromised in germ-free mice or animals treated with antibiotics. These findings represent a paradigm shift in our understanding of the mode of action of many compounds having an impact on the host-microbe mutualism (Visual 5, Science 2013). These findings have been extended to platinum salts (oxaliplatin, cis-platine) as well as to a combination of anti-IL-10R mAb+CpG for Ilda et al. Science Nov 2013 (Trinchieri’s group at the NIH, USA).

2. **Context of CTLA4 blockade:** The immune checkpoint blocker (ICB) anti-CTLA4 Ab is a first-in-class compound approved for reinstating cancer immunosurveillance and prolonging survival in metastatic patients. However, this clinical benefit is often associated with immune-related side effects at sites exposed to commensal flora such as the large intestine. Uncoupling efficacy from toxicity is a challenging issue for the future development of ICB. Her team showed (and submitted to Science) that the antitumor effects of CTLA4 blockade, largely dependent upon Toll like receptor (TLR2/TLR4 receptors, markedly rely on the regulatory commensal Bacteroides fragilis (Bf) (in coordination with Bacteriobacteria cenocepacia). Innate signaling induced by specific TLR2/TLR4 agonists failed to compensate the lack of tumoricidal activity mediated by CTLA4 blockade in germ free (GF) or antibiotics-treated mice while the IL-12-dependent cognate immunity directed against Bf could do so. Hence, anti-CTLA4 Ab elicited protective Bf-specific Th1 immune responses in specific pathogen free (SPF) mice that could be substituted, in GF animals, by oral Bf-purified Bf-associated polysaccharides or a Bf-specific adoptive T cell transfer, without triggering overt colitis. Ipilimumab could also restore Bf-specific Th1 immune responses in a fraction of advanced melanoma patients. This study unravels the key role of Bf fragsilis in the immunostimulatory effects of anti-CTLA4 Ab, opening up novel strategies to safely broaden its clinical efficacy (Vezzoni et al. Science Nov. 2015). At the same time, Cajewski’s group in Chicago showed that Bifidobacteria from the gut influence the tumor microenvironment in such a way that anti-PDL-1 Ab can induce a prominent anticancer immune response (Sivan et al. Science Nov. 2015).

- **Setting of PD-1/PDL-1 blockade:** In September 20 2017, the demonstration of the deleterious role of antibiotics in the clinical efficacy of PD-1 blockade in lung, kidney and bladder cancer patients was brought up, highlighting the role of Akkermansia muciniphila as the main player in the immunomodulatory effects of pembrolizumab or nivolumab (Routy et al. Science 2017 Nov2). The mechanisms by which A. muciniphila restores gut dysbiosis will be discussed, involving CCR9 and IL-12.

From these findings, we infer that oncomicrobiotics and/or fecal microbial transplantation could be considered as adjuvants to the current oncological armamentarium in dysbiotic cancer bearers.

**KL08 The maintenance and mobilization of resident immune memory cells**

**KL08.1 The maintenance and mobilization of resident immune memory cells**

**A. Radbruch**
1. Deutsches Rheumaforschungszentrum, Berlin, Germany. 2. Charité University Medicine, Berlin, Germany.

Population of memory T lymphocytes and memory plasma cells residing in epithelial tissues and in the bone marrow provide first-line protection and longterm memory to prevailing antogenic challenges of the environment. We have now also identified memory B lymphocytes of the bone marrow as a population distinct from their splenic counterparts in terms of repertoire and phenotype. Apparently the resident memory lymphocytes are not maintained by homeostatic proliferation. For memory plasma cells of the bone marrow, we could demonstrate that they are maintained individually by stromal cells. Their survival is dependent on cell to stromal cell, inducing PI3K signaling, and on the cytokines April or BAFF from their environment, inducing NFkB signaling. In synergy, both signaling pathways in plasma cells upregulate expression of the vital transcription factor IRF4 and prevent caspase-induced apoptosis. Memory T and B lymphocytes of the bone are maintained individually on stromal cells as well, and sensitive to inhibition of the PI3K pathway, suggesting that stromal cells play a pivotal role for immunological memory, by inducing vital, cell-contact dependent PI3K signaling. In secondary immune reactions, resident quiescent T and B lymphocytes obviously have to be mobilized from their memory niches. We could show for resident CD4+ memory T lymphocytes that this mobilisation results in amplification of the specific memory lymphocytes, and (b) to the emigration of specific resident memory T lymphocytes into the blood, and their participation in the secondary immune reaction.
S.A2.01 Dissecting blood and immune cell lineages by endogenous barcoding

H. Rodewald, W. Pei, T. Feyerabend, K. Klapproth, K. Busch, A. Schuon, T. Benz, X. Wang, J. Rössler, T. Höfer; German Cancer Research Center (DKFZ), Heidelberg, Germany.

Deconvolution of cell lineage origins and relationships remains a major challenge. Once achieved, this would allow deep insights into understanding not only the formation and maintenance of tissues but also of stem and progenitor cell fates and clonally organized developmental pathways. High-content immunofluorescent image analysis has revolutionized the study of hematopoietic cell lineages and has also generated large datasets, which are often difficult to interpret. To address this challenge, we have developed a novel endogenous genetic fate mapping system that utilizes Cre-recombinase-dependent substrate in cells in vivo (Pei, Feyerabend et al. Nature 2017). We have introduced barcodes in embryonic HSC progenitors and in adult HSC and studied the lineage outputs from HSC clones, as well as their sizes in the bone marrow. The data demonstrate common myeloid-erythroid, and common lymphocyte pathways as fundamental structures of the hematopoietic system. These endogenous barcoding studies are now exploited to unravel at high resolution lineage relationships of tissue resident macrophages of embryonic origin.

S.A2.02 Implications from monogenic autoimmunity

K. Kisand; University of Tartu, Tartu, Estonia.

Autoimmunity caused by single gene defects is a rapidly growing group of hereditary diseases. Autoimmune tissue damage is often accompanied by susceptibility to certain infectious agents highlighting the dual function of the immune system - to recognize pathogens and secure tolerance to own tissues. APECED (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy) is caused by AIRE gene mutations resulting in defective central T cell tolerance. However, the most striking feature of APECED patients is the presence of multiple extra-tissue autoantibodies that target cytokines: type I interferons and Th17 cytokines. Monoclonal auto-antibodies isolated from patients’ B cells show extremely high affinity which has permitted their application for diagnostic and potentially therapeutic purposes. STAT1 gain-of-function (GOF) mutation is another gene defect that leads to chronic mucocutaneous candidiasis and variable manifestations of autoimmunity. In these patients the development of Th17 cells, that are indispensable for fungal protection, is impaired. The precise molecular mechanisms causing STAT1 signalling imbalance in STAT1 GOF cells are still unclear. We have applied ChIP-Seq to shed some light on these processes. In spite of the rarity of monogenic autoimmune diseases their impact on unfolding of the processes that shape and regulate the immune responses is immense.

S.A2.03 Strategies for enhancing human immunity during ageing

A. N. Akbar; University College London, London, United Kingdom.

Older people suffer from increased incidence and severity of infections and also cancer. In addition, vaccination therapies becomes less efficient with age. It is possible to enhance the function of immune cells during ageing in both humans and mice by blocking the function of a group of proteins called the steins (Lanna et al Nature Immunology 2017). The steins were found to form a molecular complex, termed sMAC (stein-induced MAPK activation complex), with p38 and also the other two classes of MAPKs namely ERK and JNK in these cells. This for the first time provides a mechanism for integrated activation of all three classes of MAPK within a single cell type. The inhibition of steins in human T cells or in genetically modified mice that are stein-deficient, enhanced the function of T lymphocytes. Moreover, when old stein deficient mice were vaccinated against influenza, the response was considerably enhanced as compared to old stein replete animals. Therefore temporary immunity enhancement by short-term inhibition of steins could boost immune function that could be beneficial during anti-ageing immunotherapy, for instance during vaccination of older humans.

S.A3 Immunomonitoring and biomarkers

S.A3.01 CD Maps - antigen density measurements of CD1-CD100 on human lymphocytes and thymocytes

T. Kalina; I. Stuchlý, D. Kužílková, K. Filer, M. Cuenca, E. Blanco Álvarez, S. J. W. Bartol; Monash University, Melbourne, Australia.

Leucocyte receptors have been characterized using antibodies validated by the human leucocyte typing HLA/HCDC organization (www.hcdm.org). However, expression information is outdated or incomplete. CD Maps project aims at quantitative mapping of expression of all CD molecules across the spectrum of leucocyte subsets. We measured PE conjugated CD1-CD100 antibodies in the context of four standardized 8-color panels on cells from three tissues (blood, thymus and tonsils). We attempted to re-map CD markers expression in thymocytes using a 37 marker mass cytometry panel (29 surface markers and 8 transcription factors). We expert gated 9 thymocyte stages based on canonical markers (CD34, CD1a, CD3, CD4, and CD8). We developed fast time and paths inference algorithm, which involves branching and also accounts for possible alternative developmental paths. We processed the thymocytes through computational algorithm in parallel to expert gating. The simulated paths reached the canonical CD4 helpers end in 79% and the canonical CD8 end in 19% respectively. The CDMaps project generates a broad and updated online database containing the expression profiles of all CD markers on human leukocyte subsets present in blood, tonsil, and thymus. Quantitative information on receptor expression is important for mechanistic studies as well as flowcytometric panel design and design of novel biological therapeutics. Therefore it will serve as a useful resource to guide and advance therapies into basic, translational and clinical immunology.

S.A4 Germlinal centers and B cell differentiation

S.A4.01 Germinatal centers under the lens: Differentiation of plasma cells and memory B cells from germinatal center precursors

R. Brink; 1Garvan Institute, Darlinghurst NSW, Australia, 2UNSW Australia, Darlinghurst NSW, Australia.

Germinatal centers form in secondary lymphoid organs in response to challenge with T-cell-dependent antigens. After extrafollicular and follicular interactions with cognate antigen and T helper cells, responding B cell clones can enter the germinatal center response, where they undergo iterative cycles of proliferation, somatic hypermutation and selection such that clones acquire increased affinity for the eliciting antigen preferentially accumulate. This process is also associated with the differentiation of long-lived memory B cells and antibody secreting plasma cells from germinatal center B cell precursors. These two populations facilitate long-term immunity against infectious pathogens and underpin the efficacy of almost all current vaccines. Although the importance of memory B cells and plasma cells to long-term immunity is well established, the processes that regulate their production during the germinatal center response have been difficult to identify due to the dynamic nature of the germinal center and the difficulty of tracking the antigen specificity and fate of responding B cells. We have used a high resolution in vivo model in which B cells respond to a low affinity variant of the model antigen hen-egg lysozyme, to identify the precursors of both memory B cells and plasma cells in the germinatal center. A fundamental dichotomy in the differentiation of these two critical germinatal center outputs was revealed in terms of both antigen affinity and cell cycle status. This system not only optimises the specificity and potency of the antibody response but provides response flexibility within the long-term memory B cell pool.
S.AS 01 Initiation of immune responses

G. Guarda*
IRB, Bellinzona, Switzerland.

Natural Killer (NK) cells are cytotoxic lymphocytes that contribute to the elimination of virally infected or transformed cells. Their cytotoxic activity is regulated by two complementary sets of receptors; the activating receptors, which are engaged by stress-induced molecules, and the inhibitory ones, recognizing MHCClass I molecules. NK lymphocytes are extremely powerful and several efforts are undertaken in order to harness their full potential for therapeutic purposes. A deeper understanding of the mechanisms regulating their development, survival, and activity, is therefore needed. Recently, the study of mechanisms specifically regulating their activation as well as their metabolism has revealed new pathways and players relevant to NK cell-mediated immunity. Advances in our understanding of such mechanisms and the consequences on innate cytotoxic immunity will be discussed.

S.AS 02 dendritic cells in HIV-1 sensing and restriction

T. B. H. Geijtenbeek1,2,3,4
1Amsterdam UMC, Amsterdam, Netherlands, 2Amsterdam Infection & Immunity Institute, Amsterdam, Netherlands.

Sexual transmission is the primary route of infection by HIV-1 and mucosal dendritic cell (DC) subsets are amongst the first targets for HIV-1. Although DCs are paramount to the induction of antiviral immunity to HIV-1, it is becoming evident that HIV-1 subverts DCs for dissemination to T cells as well as escape from antiviral immunity. HIV-1 productively infects submucosal DCs but this does not lead to an antiviral type I Interferon (IFN) immune response as HIV-1 escapes viral sensing in DCs. We identified the dead-box helicase DDX3X as a RNA sensor for HIV-1 and our recent data strongly suggest that HIV-1 blocks this viral sensor in DCs, preventing triggering of antiviral immunity. Strikingly, interfering with the inhibitory pathway, leads to efficient DC maturation, type I IFN and cytokine responses, which limits HIV-1 replication in vitro and in vivo. Here, we will discuss the importance of DDX3X in sensing HIV-1 replication and induction of innate and adaptive immunity. Furthermore, we will discuss how synthetic DDX3 ligands can be used as adjuvants in immunotherapy to induce innate and adaptive immune responses. However, not all DC subsets become infected by HIV-1 as our data show that mucosal Langerhans cells (LCs) are resistant to HIV-1 infection. LCs efficiently capture HIV-1 and route the virus to pre-defined degradative pathways, which prevents infection. Here I will discuss the molecular mechanisms underlying the distinct functions in DC subsets in HIV-1 infection and how we can harness these mechanisms to prevent infection and enhance antiviral immunity.

S.AS 03 The role of complement in Tolerance

M. Botto
Imperial College London, London, United Kingdom.

Complement has been shown to contribute to the immunopathology of several autoimmune diseases including systemic lupus erythematosus (SLE). Paradigmatically, however, complement also appears able to protect against autoimmunity since complement deficiencies, particularly C1q deficiency, strongly predispose to the development of SLE. There are currently several proposed mechanisms whereby deficiency or low levels of complement might lead to break of tolerance, and these are not mutually exclusive. One of the hypotheses to explain the heightened susceptibility to the development of SLE in the absence of C1q invokes an important role for complement in the waste-disposal mechanisms of dying cells. However, impaired clearance of such cells is, on its own, insufficient to induce autoimmunity. The data available from knockout mice emphasize that the break of tolerance depends on many factors in addition to the defective removal of dying cells. Recent findings have highlighted that C1q and C3 can modulate both adaptive and innate immune responses. In addition, C1q may not only act as initiator of the classical complement pathway, but can also mediate multiple immune responses in a complement activation independent manner. In particular, C1q can restrain autoimmunity by acting as a metabolic regulator of effector CDC T cells. In summary, the traditional view of the role of complement in tolerance needs revision as evidence is emerging of an important interplay between complement and immunometabolism in autoimmunity.

S.B1 Tumor vaccination principles and immunotherapy

E. Vivier
Aix Marseille Univ, CNRS, INSERM, APHM, CIML, Inate Pharma, Marseille, France.

Immuno-oncology, including checkpoint inhibitors targeting the PD-1/PD-L1 (PD-x) axis in particular, has revolutionized cancer treatment. However, only a subset of patients respond to these therapies, and the development of drug resistance is frequent. Here, we report that the blocking of the inhibitory NKG2A receptor enhances tumor immunity by promoting both Natural Killer (NK) and CDC T-cell effector functions in mice and humans. Monalizumab, a humanized anti-NKG2A antibody, enhanced NK cell activity against various tumor cells and rescued CDC T-cell function in combination with PD-x axis blockade. Monalizumab also stimulated NK-cell activity against antibody-coated target cells. We also established proof-of-principle for the use of combined immunotherapy with monalizumab and cetuximab in a phase I clinical trial, in which the combination showed promise for the treatment of patients with squamous cell carcinoma of the head and neck (SCCHN). NKG2A targeting with monalizumab is thus a novel checkpoint inhibitor mechanism promoting anti-tumor immunity by enhancing the activity of both T and NK cells, which may complement the first-generation immunotherapies against cancer.

S.B1 03 Intratumorally produced immunoglobulin repertoires

D. M. Chudakov1,2,3
1Institute of Bioorganic Chemistry, Moscow, Russian Federation, 2Privatklinik Research Medical University, Nizhny Novgorod, Russian Federation, 3Central European Institute of Technology, Brno, Czech Republic.

The emerging data shows that B cells may play an essential role in the immune response to cancer - as antigen-presenting cells, and by cytokines and antibodies production. Been present at limited counts in a tumor infiltrate, B cells may participate in generation of tertiary lymphoid structures, convert to the plasma cell phenotype and produce huge amounts of antibodies, which specificity and isotype-determined functional activity may influence on either cancer surveillance or immunosuppression. From this point, repertoire of intratumorally produced antibodies represents yet poorly explored component of cancer-immunity interaction that could play its role as a biomarker or as a source of tumor-specific receptors for precise immunotherapy. I will briefly summarize current knowledge on tumor-infiltrating B cells and immunoglobulin repertoire they produce, complementing it with our recent experience on antibody repertoire profiling with high-throughput sequencing. In particular, I will cover full-length nearly error-free immunoglobulin repertoire profiling using 5'RACE with UMI, extraction of immunoglobulin repertoires from RNA-Seq data with MiXCR, and provide guidelines for repertoire diversity/clonality analysis. Research supported by grant of the Ministry of Education and Science of the Russian Federation 14.W03.31.0005.

S.B2 Environmental regulation anti-tumor responses

K. E. de Visser
the Netherlands Cancer Institute, Oncode Institute, Amsterdam, Netherlands.

Metastasis formation is a key challenge in cancer patient care that urgently needs solutions. It is now well established that cells and mediators of the immune system influence these processes. Historically, our immune system has been considered to form an intrinsic defense mechanism against cancer and metastasis. Yet, the majority of cancer types exploit a myriad of strategies to successfully evade detection by the immune system. In fact, mounting evidence supports the notion that cancer cells hijack the immune system for their own benefit, allowing them to escape from immune attack, maintain limitless proliferation, survive under dire circumstances and spread to distant organs. The overall goal of our research is to understand how the immune system influences metastasis formation. To achieve this, we utilize pre-clinical mouse models that faithfully recapitulate human breast tumorigenesis in combination with immune profiling studies in breast cancer patients. We have previously discovered that breast tumors elicit a systemic inflammatory cascade to dampen anti-tumor T cells and promote metastasis formation. Current efforts are underway to dissect how the genetic make-up of breast tumors dictates activation of systemic immunosuppressive inflammation. Our findings provide novel mechanistic insights into the thus far poorly understood metastatic cascade, and open new avenues for the development of therapeutic strategies to unleash anti-tumor immunity and to inhibit metastatic disease.
SYMPOSIA

S.B3 The Yin and Yang of T-cell regulation

S.B3.03 Maintaining T cell tolerance via the CTLA-4 pathway

L. S. K. Walker; University College London, London, United Kingdom.

The immune system provides vital protection from infection and cancer but needs to be tightly regulated to prevent the development of autoimmune diseases such as type 1 diabetes and rheumatoid arthritis. The ability to augment or diminish the immune response in a controlled fashion holds the promise of boosting anti-tumour responses or silencing autoimmune diseases respectively. A major checkpoint controlling immune responses involves the T cell molecule CTLA-4 that is expressed at high levels in FoxP3+ regulatory T cells. CTLA-4 controls engagement of the T cell costimulatory receptor, CD28, by binding to their shared ligands and removing them from antigen presenting cells by a process of trans-endocytosis. Our lab seeks to understand how this mechanism operates in the steady state to maintain tolerance and allow appropriate immune responses to develop in a timely manner. Understanding the molecular basis of the CTLA4 checkpoint will ultimately empower us to manipulate the immune response in a more precise manner.

S.B4 T cell activation and exhaustion

S.B4.02 Immunodeficiencies associated with increased T cell senescence


Activated PI3Kδ syndromes 1 and 2 (APDS1 and 2) are primary immunodeficiencies caused by either gain-of-function mutations in the PIK3CD gene encoding p110δ (the catalytic subunit of PI3Kδ) or heterozygous mutations in the PIK3R5 gene encoding p55α and p50δ (regulatory subunits of PI3Kδ). The disease causing mutations lead to hyper-activated PI3K-δ signalling in lymphocytes. APDS1 and 2 are combined immunodeficiencies with variable clinical phenotypes. The clinical symptoms include recurrent respiratory infections, bronchiolitis, lymphophagia and hypogammaglobulinemia frequently associated with elevated IgM serum level. Both diseases appear to predispose to B cell lymphomagenesis especially diffuse B cell lymphoma and Hodgkin lymphoma. Abnormalities of B lymphocyte subpopulations, e.g. B cell lymphopenia and higher frequency of transitional B cells, and of T cell subpopulation, e.g. decreased number of naive CD4 and CD8 T cells and increased frequency of effector/effector memory CD8 T cells and CCR6+ (positive) T cells, are frequently observed in both diseases. Immunoglobulin replacement therapy, rapamycin, allogeneic hematopoietic stem cell transplantation and selective PI3Kδ-specific inhibitors (currently on clinical trials) are possible treatment options. The work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Agence Nationale de la Recherche as part of the "Investissement pour le Futur" program: ANR-10-IAHU-01 and by ANR-15-CE15-0020 (ANR-PiKimmun), the Ligue Contre le Cancer - Comité de Paris, the Fondation ARC pour la recherche sur le Cancer, and the Centre de Référence Déficits Immunologiques Héréditaires (CEREDIH).

S.B4.03 Maintenance of exhaust T cells in chronic infection

D. Zehn; Munich, Germany.

Failure to clear an infection can coincide with the appearance of T cells which express low amounts of cytokines and high levels of inhibitory receptors (PD-1, Ldg-3, Tim-3). This phenotype has so far been viewed to mark exhausted and terminally differentiated effector cells. Nonetheless, “exhausted” T cell populations in chronic infections and tumors are long-living and can be expanded upon blocking inhibitory receptor ligand systems. This raised the questions how these populations are maintained and which mechanisms act in case of therapeutically induced re-expansion. We and others identified recently that both long-term T cell maintenance in chronic infections and re-expansion following blockade of PD-1 signaling are depending on a small subpopulation of T cells, which express the transcription factor Tcf-1. We showed that Tcf-1+ positive T cells share key features with conventional memory T cells while they co-express markers of “exhausted T cells” (i.e. PD-1). These memory-like T cells are capable of undergoing self-renewal while they are continuously generating terminally differentiated effector cells. Given the presumed central role of the memory-like population for therapeutic purposes, we will report recent advances on molecules and mechanisms that control size and function of this subset.

S.C1 Maintenance and local regulation of tissue specific immunity

S.C1.02 Functions of Resident Memory T Cells

D. Masopust; University of Minnesota, Minneapolis, MN, United States.

Resident memory T cells, often abbreviated T RM, occupy tissues without recirculating and provide a first response to infections reencountered at body surfaces, where they accelerate pathogen clearance. T RM also likely play critical roles in tumor immunosurveillance, and may contribute to immune disorders, allergies, and autoimmunity. This talk will share recent and ongoing investigations of T RM function. Evidence will be presented that T RM are capable of autonomously regulating the expansion of local immunosurveillance independently of central memory or proliferation in lymphoid tissue. Data will be communicated that reveal a nonlymphoid origin of secondary lymphoid organ T RM and that suggest vaccination strategies by which memory CD8 T cell immunosurveillance can be regionalized to specific lymph nodes. Experiments supporting developmental plasticity among T RM will be reviewed. Lastly, evidence will be presented that natural antiviral T RM can be repurposed to control or eliminate tumors.

S.C1.03 Activation of Ca2+ regulated pathways downstream of Pattern Recognition Receptors

F. Granucci1,2; L. Marongiu1,2; J. Artuso1, F. Mingozzi1, J. Zanoni2; 1Milan, Italy; 2University of Milano-Bicocca, Milan, Italy.

Microenvironmental invasions are perceived by pattern recognition receptor (PRR)-expressing cells of the innate immune system. Among PRRs, TLRs and their co-receptors are the best characterized. CD14, with LPS binding protein (LBP), TLR4 and MD-2 forms the multi-receptor complex for LPS. CD14 is a glycosylphosphatidylinositol (GPI)-anchored protein abundantly expressed on DCs and macrophages. CD14 concentrates the LPS signal, mediates the relocation of TLR4 and MD2 to the endosome for the initiation of the TRIF signaling pathway and is responsible for Ca2+ mobilization and NFAT signaling pathway activation in DCs. Ca2+ mobilization is one of the first events for the initiation of the NFAT signaling pathway. We have investigated the mechanism of Ca2+ mobilization leading to NFAT activation in myeloid mouse and human DCs following LPS stimulation. Following LPS stimulation, IP3, second messenger induces a SOCE through IP3 receptor 3 channels co-localized with CD14. For this process to occur, Ins(1,3,4,5)P4, generation by the IP3 kinase (ITPK) B is required to antagonize IP3 dephosphorylation and increase IP3 availability. ITPKs pharmacological inhibition restrains inflammatory events (such as increased vessel permeability or inflammatory arthritis) regulated by NFATs in the presence of LPS, similar to the direct inhibition of NFATs by nanobodies. ITPKB represents a new target for anti-inflammatory therapies aimed at inhibiting specific DC functions. The NFAT-controlled phenomena and the consequences of NFAT activation in innate immune cells will be discussed in models of microbial infections and sterile inflammation.

S.C2 Immune signalling and therapy in autoimmunity

S.C2.01 Myeloid and glial cells collaborate to regulate neuroinflammation

T. Owens1, G. Webster2, R. Khorooshi3, J. Marczynska1, A. Wlodarczyk1, A. Benmamar-Badel3, R. Dieu1; 1University of Southern Denmark, Odense, Denmark; 2Innate Immunotherapeutics, Auckland, New Zealand.

Tissue macrophages and blood-deducted regulatory myeloid cells play important roles in development and normal homeostasis. In the neonatal central nervous system (CNS), a subset of tissue-resident CD45+CD11b+ microglia that express CD11c dramatically expand, and are a critical source of myelogenic GFI1. Transfer of such cells to adult mice alleviates experimental autoimmune encephalomyelitis (EAE) which identifies an anti-inflammatory role for this microglial subset in the developing CNS, whose mechanism remains to be established. We have also examined how innate signaling can direct anti-inflammatory myeloid cell programs in the adult CNS. Microglia as well as extraparenchymal CD45+CD11b+ macrophages were induced to produce interferon-alpha and -beta by intrathecal TLR3/4-RIG-I (Igand) poly-I:C, and this alleviated EAE. Intrathecal administration of a bi-specific NOD2 and TLR9 microparticle [MS416] induced marked influx from blood of CD45+GR-1+CD11b+CD11c+ monocytes as well as CD45+GR-1+Ly6G+CD11b+CD11c+ neutrophils, as early as 2h after injection.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 16
Both populations phagocytosed MS1461, produced IFNβ and expressed PD1L. Intrathelial MS1461 alleviated EAE and this was dependent on IFNAR signalling. RNAseq analysis showed upregulation in the CNS of NFκB, Jak-STAT, TLR and NLR signalling pathways, as well as Nfngamma and neuropoietin- (CXCL1, CXCL2) and monocyte-attracting (CCL2, CXCL10) chemokines. Microglia were a prominent source of chemokines. These studies collectively show that CNS-intrinsic signals promote and recruit endogenous and exogenous myeloid programs that collaborate to maintain CNS homeostasis in development, and in response to infection or tissue damage.

S.C.02.03

How to modulate TCR signalling for peptide therapy in autoimmunity

D. C. Wrath;
University of Birmingham, Birmingham, United Kingdom.

Control of autoimmune and allergic conditions can be reinforced by tolerance induction with peptide epitopes; this presentation will focus on the mechanisms involved. Peptides must mimic naturally processed epitopes, and be designed to target tolerogenic, steady-state dendritic cells (DCs). Steady-state DCs express low levels of costimulatory molecules and induce ‘abductive’ activation of T cells whereby T cells undergo initial cell division but do not differentiate. CD4+ T-cells become anergic following their first encounter with peptide. This depends on strength of signal via the T cell receptor (TCR) and the balance between TCR and costimulatory signalling. Continuation of peptide therapy results in the generation of anergic, IL-10 secreting CD4+ T-cells with regulatory function. The loss of proliferative capacity correlates with a cytokine switch from a pro-inflammatory to a phenotype characterised by secretion of the anti-inflammatory cytokine IL-10. The IL-10 secreting Tr1-like cells suppress dendritic cell maturation, prevent Th1 cell differentiation and create a negative feedback loop for Th driven immune pathology. This mechanism leads to bystander suppression whereby Tr1-like cells specific for one antigen can suppress T cells specific for other antigens derived from the same tissue. Tolerance induction involves upregulation of transcription factors controlling IL-10 and inhibitory receptors limiting T cell signalling. The peptide therapy approach is a highly selective approach for prevention and treatment of autoimmune diseases in humans. Results from clinical trials of peptide immunotherapy in multiple sclerosis and Graves’ disease will be described.

S.C.03

Transplantation

S.C.03.01

Treating autoimmune diseases with T regulatory cells - first clinical data

P. Tzonkowki;
Medical University of Gdańsk, Gdańsk, Poland.

T regulatory cells (Tregs) are considered a viable option in tolerance induction treatment in the clinic. First promising clinical experiments and trials with clinical-grade Tregs cultured as advanced therapy medicinal product (ATMP) are completed already. We will present long-term results (up to 5 years follow up) as well as our ongoing trials with Tregs in type 1 diabetes and multiple sclerosis discussing metabolic and immune background of the patients, which, in our opinion, influenced the efficacy of this treatment. In vivo results will be supported with in vitro and animal models showing activity of Tregs in auto- and allogeneic settings. References: J Transl Med. 2016;14(1):332, Diabetes Care. 2012;35(9):1817-20; Ann Surg. 2011;254(3):512-8; Clin Immunol. 2009;133(1):12-6

S.C.03.03

Transplantation, T cell engineering

C. Bonini;
UNIVERSITÀ VITA SALUTE SAN RAFFAELE, Milan, Italy.

Transplantation, T cell engineering

Chiara Bonini, Università Vita-Salute San Raffaele Ospedale San Raffaele Scientifico Institute Milano

Adoptive T cell therapy represents an innovative and promising therapeutic approach, which relies on the ability of T lymphocytes to recognize and destroy specific targets on microbes or tumors through their T cell receptors (TCR), to obtain efficient killing of cancer cells. Ideally, adoptively transferred T cells should be: 1. Specific for tumor antigens, 2. Able to expand and persist long enough to mediate a long lasting clinical response, 3. Able to counteract the immunosuppressive tumor microenvironment. TCR gene editing represents a suitable approach to generate large numbers of tumor specific T cells. The core of this approach is the transfer in patients’ T cells of genes encoding for rare tumor-specific TCR. The simple transfer of tumor specific TCR genes into T cells is affected by some limitations: genetically modified T cells shall express four different TCR chains, that might mispair, leading to unpredictable toxicity and to an overall dilution of the tumor specific TCR on lymphocyte surface, thus limiting the efficacy of therapeutic cellular product. To overcome these issues, we can adopt a TCR gene editing approach, based on the concomitant disruption of the endogenous TCR genes and introduction of the tumor specific TCR genes. Different protocols to generate high numbers of TCR edited memory stem T cells and central memory T cells, able to overcome the immunosuppressive tumor microenvironment will be discussed.

S.C.04

Manipulation of tolerance

S.C.04.01

Engineered Dendritic Cells to re-establish Antigen-Specific Tolerance in T-cell Mediated Diseases

S. Gregori;
San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), Milan, Italy.

The design of novel approaches to control antigen (Ag)-specific pathogenic T cell responses and restore tolerance represents an ambitious goal for the management of autoimmune diseases. The prominent role of dendritic cells (DC) in promoting T-cell tolerance and the development of methods to generate clinical grade products allowed the clinical application of tolerogenic DC-based therapies for the control of unwanted immune responses. The concluded clinical trials demonstrated the safety and feasibility of this approach. However, the stability of the infused DC products and the maintenance of their tolerogenic properties in vivo remain open issues to be tackled for improving the safety and the efficacy of DC-based cell therapies. Our hypothesis is that infusion of tolerogenic DC genetically modified by newly developed tolerogenic lentiviral vectors (LV) encoding autog-Derived epitopes (tolLV-DC) will promote the in vivo generation of Ag-specific tolerance via down-regulation of autoAg-specific pathogenic T cell responses and induction of long-living autoAg-specific Tregs. To this aim we developed LV-platforms that allow the expression of specific autoAg epitope and pro-tolerogenic molecules. Our preliminary data show that tolLV-DC can modulate T cell responses both in vitro and in vivo that infusion of tolLV-DC dampens Ag-specific T cell responses in vivo. The success of our strategies will help designing a safer tolerogenic DC-based cell therapy, abrogating the boosting of autoimmunity, and to stably preserve the tolerogenic properties of in vivo transferred DC.

S.C.04.02

The role of cell metabolism in Treg biology and function

B. Salomon, R. Vallion, J. Divoux, S. Greigote, E. Ronin;
CIMiP-Paris, Sorbonne Université, Inserm, CNRS, Paris, France.

FoxP3 regulatory T cells (Treg) play a major role in regulation of immune responses. Cellular metabolism of conventional T cells (Tcon) has been intensely studied revealing, among other findings, that their activation requires a switch from AMPK to mTOR driven metabolic pathways. We know little about cell metabolism on Treg homeostasis and function and published data are quite controversial. Thus, we have investigated the role of AMPK (coded by Pkhr) and mTOR in Treg by generating mice that have a conditional knockout of these molecules specifically in Treg. Whereas the FoxP3lox/lox x Pkhr-lox (AMPK&Reg) mice looked healthy, the FoxP3lox/lox x mTor-lox (mTOR&Reg) mice developed a systemic inflammatory disorder with massive immune cell infiltration, activation of Tcon, increased levels of inflammatory cytokines and immunoglobulins and died by 10 weeks of age. Interestingly, mTOR&Reg mice had increased Treg frequency in lymphoid organs but decreased Treg frequency in non-lymphoid organs, which was correlated with lower proliferation, migration and stability of mTOR-deficient Treg. In the heart of a cancer model, tumor growth was reduced in these mice, which was correlated with decreased proportion of tumor-infiltrating Treg and higher activation of Tcon. Thus, AMPK seems critical for the homoeostasis of Treg infiltrating tumors but dispensable at steady state. Our results reveal a new role of the AMPK/mTOR metabolic balance in Treg biology.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Tolerogenic dendritic cells as a therapeutic strategy to induce tolerance in multiple sclerosis

E. Martinez-Caceres,
Germsnis Trias i Pujol Hospital. Universitat Autonoma Barcelona, Badalona (Barcelona). Spain.

Multiple sclerosis (MS) is a chronic, inflammatory, and neurodegenerative disease of the central nervous system. Its prevalence is increasing worldwide, and is now considered, after traumatisms, the main cause of disability in young people. Current treatments in MS do reduce disease activity, do not decrease long-term disability and progression and must be administered lifelong, causing relevant side effects. Therefore, safer and more effective treatments are needed. In this context, a promising strategy for the attenuation of pathology is to use tolerogenic dendritic cells (tolDC). Our group has developed an antigen-specific cell therapy based on autologous monocyte-derived tolDC differentiated in the presence of vitamin D3 (VitD3), loaded with a group of myelin peptides (vitD3-tolDC), to induce tolerance in MS patients. In vitro studies in co-culture experiments demonstrated a potent immune-regulatory activity of vitD3-tolDC, reducing lymphocyte proliferation and IFN-γ production and producing low levels of IL-10. Moreover, in vivo studies in the animal model of MS - experimental autoimmune encephalomyelitis - revealed a beneficial effect of vitD3-tolDC, ameliorating the severity of the disease. These pre-clinical results, as well as, reported outcomes from previous clinical trials using tolDC, have led to a Phase I/IIa clinical trial in patients with active MS as approved by the Spanish regulatory Agency (AEPMPS) which is currently ongoing (N° EudraCT: 2015-003541-26, available at ClinicalTrials.gov identifier: NCT02903537, Tolervit-MS).

Allergy, asthma and therapy

Spontaneous protein crystallization in asthma: a new pathway for intervention

B. Lambrecht,* E. Persson,†
1VIB, Immunology Research Center, Ghent, Belgium; 2VIB, Ghent, Belgium.

Asthma is a chronic inflammatory airway disease rich in eosinophils. As early as 1853, Charcot and von Leyden described extracellular deposits of morphologically diverse crystals in airways of asthmatics. Charcot-Leyden crystals (CLC) are made from Galactin-10 (Gal10), one of the most abundant yet least understood proteins in eosinophils. A pathogenic role for CLC or Gal10 in airway inflammation has not been established. Here, we show that the ex vivo crystal packing interface of CLC obtained from the upper airways of patients is identical to recombinant Gal10 crystals, and could be mutated to prevent auto-crystallization. Only in the crystalline state could Gal10 induce airway inflammation. Remarkably, Gal10 crystal packing contacts between Gal10 co-administered with harmless antigens could stimulate humoral and cellular immunity, promoting TH2 sensitization and airway eosinophilia in vivo in a mouse model. To target this type of crystal-induced inflammation in humans, we generated llama antibodies against crystalline Gal10 that rapidly dissolved preformed Gal10 crystals and CLC in the mucus of patients. In a humanized severe combined immunodeficiency model of allergic airway inflammation, administration of crystal dissolving antibodies suppressed allergen-induced lung inflammation, human IgE synthesis and airway mucin production. As a mechanism of action, the crystal dissolving antibodies targeted the key crystal packing contacts underlying the intrinsic auto-crystallization behavior of Gal10. Together, our data demonstrate that beyond serving as markers of eosinophilia, CLC actively promote inflammation and adaptive immunity. As protein crystallization is reversible by antibody treatment, antagonizing the crystalline state of pro-inflammatory proteins is a new intervention strategy.

Isotype-specific regulation of BCR signal transduction

J. Wieneands, N. Engels, M. Engollke,
Institute for Cellular & Molecular Immunology, University Medical Center Göttingen, Göttingen, Germany.

The role of the B cell antigen receptor (BCR) in triggering antibody-mediated immune responses is well established. However, it has become clear more recently that BCR ligation does not deliver an all-or-nothing signal for B cell activation. Multiple levels of BCR intrinsic fine tuning exist that can amplify or attenuate the primary ‘Go Signal’ provided by phosphorylated Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) and the associated kinase Syk. While ITAMs reside in the cytoplasmic segments of the canonical BCR signal elements, Ig-a and Ig-β, a second phosphorylation module is accommodated in membrane-bound IgG and IgE on class-switched memory B cells. It is called the Ig Tail Tyrosine (ITT) motif and recruits the versatile signaling protein Grb2. The phospho-ITT/Grb2 axis provides a co-stimulatory signal that renders antigen-experienced B cells less dependent on T cell help during recall responses. Indeed, memory B cell responses are dominated by class-switched IgG isotopes, most notably IgG1. Given the anaphylactic properties of IgG antibodies, activation of IgG-positive B cells is tightly regulated. The long isoform of membrane-bound IgE on human B cells encompasses an ER retention motif in its extracellular membrane-proximal domain that restricts surface IgE-BCR expression and concomitant downstream signaling events. BCR-autonomous signal inhibition can also be brought about by linking up with negative regulatory modules such as lipid phosphate SHIP known to limit BCR activation through inhibitory coreceptors. In summary, the BCR can regulate its signal output by various mechanisms to promote protective humoral immunity or keep potentially harmful B cells in check.

Genetic restriction of antigen-presentation dictates allergic sensitization and disease in humanized mice

A. Neunkirchner,1 B. Kratzer,1 C. Kühler,1 U. Smole,1 M. Luger,1 K. Schmetterer,1 D. Trajpin,1 V. Leh-Reichl,1 E. Rasloniec,1 R. Naumann,1 L. Kenner,1 B. Jahn-Schmidt,1 B. Bahlke,1 R. Valentov,1 W. Pickl,*
1Medical University of Vienna, Vienna, Austria; 2Memphis Vetrans Affairs Medical Center, Memphis, TN, United States; 3Max Planck Inst Molecular Cell Biology Genetics, Dresden, Germany.

IgE-associated allergies result from misguided immune responses against usually innocuous environmental or food antigens. CD4+ T lymphocytes are critical for initiating and perpetuating that process, yet the crucial factors determining whether an individual gets allergic are largely unknown. We here created a novel human TCR and HLA-DR1 (TCR/ DR1) transgenic mouse model of asthma, which specifically reacts to the human-relevant major pollen allergen from Artemisia vulgaris (mugwort) to examine these critical factors upon natural allergen exposure via the airways in the absence of systemic priming and adjuvants. We discovered that acute allergen exposure led to IgE-independent airway hyperreactivity (AIR) and Th2-prone lung inflammation in TCR/DR1, but not DR1, TCR or WT control mice, that was alleviated by prophylactic IL-2/anti-IL-2 mAb complex-induced expansion of Tregs. In contrast, chronic allergen exposure sensitized one third of single DR1 transgenic mice, however, without impacting on lung function. Similar treatment led to AHR and Th2-driven lung pathology in >90% of TCR/DR1 mice. Prophylactic and therapeutic expansion of Tregs with IL-2/anti-IL-2 mAb complexes blocked the generation and boosting of allergen-specific IgE associated with chronic allergen exposure. Our findings reveal allergen-specific T effector and Treg cell frequencies next to genetic restriction of allergen-presentation, as important factors for allergy.

C6 Innate control of inflammation and tissue repair

The Yin yang of phosphatase regulation and inflammatory cytokines

A. Montovani,
Istituto Clinico Humanitas, Humanitas University, Rozzano, Italy.

The tumor microenvironment (TME) is a complex network, which includes soluble factors and components of the extracellular matrix as well as stromal, endothelial and immune cells. Immune cells and, among them, myeloid cells, play important roles in cancer development and can promote or inhibit cancer initiation and progression. Among tumor-infiltrating immune cells, macrophages are well-known determinants of cancer-related inflammation and are typically characterized by their remarkable plasticity. This consists in the ability to acquire a wide spectrum of activation states in response to various signals derived from the microenvironment. Classical M1 and alternative M2 macrophages represent the paradigm of this property. Tumor-associated macrophages (TAMs) usually display a so-called ‘M2-like’ phenotype that can foster tumor progression in different ways, namely by promoting genetic instability, angiogenesis and metastasis and by restraining anti-tumor adaptive immunity. Notably, TAMs can also play a dual role in the response to conventional anti-tumor therapies: they can enhance the anti-neoplastic effect or, in contrast, they can sustain a tumor-promoting response and so foil the anti-cancer power of these drugs. We recently identified IL-1Rβ, which we had cloned as TIRβ and is also known as SIGIRR, as a checkpoint in NK cells, which negatively regulates response to myeloid derived IL-1β. Uninfected NK cells mediate resistance to liver carcinogenesis and metastasis at NK rich anatomical sites. Thus the organ immunological context is a key determinant of the role of innate and adaptive immunity in tumor progression.
Symposia

S.C6.02 Extracellular DNA traps: neutrophils, eosinophils, basophils
H. Simon;
Institute of Pharmacology, Bern, Switzerland.

Extracellular DNA traps represent an important element of the innate immune response and they are seen in association with many infectious, allergic, and autoimmune diseases. They are able to kill bacteria and can be formed by neutrophils, eosinophils and basophils. Although the functional importance of extracellular DNA traps is generally accepted, the origin of the DNA scaffold, as well as the mechanism of their generation, remains unclear and a matter of dispute. In my presentation, I will focus on the role of Optic Atrophy 1 (OPA1), a mitochondrial inner membrane protein known for its role in mitochondrial fusion and structural integrity. Disfunctional OPA1 mutations cause atrophy of the optic nerve leading to blindness. We demonstrate that lack of OPA1 reduces the activity of mitochondrial electron transport complex I in neutrophils, which, owing to lowered NAD+ availability, causes a decline in adenosine-triphosphate (ATP) production through glycolysis. OPA1-dependent ATP production in these cells is required for microtubule network assembly and for the formation of neutrophil extracellular traps (NETs). Moreover, conditional knockout mice lacking Opa1 in neutrophils (Opa1fl/fl) exhibit a reduced antibacterial defense capability against Pseudomonas aeruginosa. Hence, these findings establish an impact of OPA1 function on the innate immune system.

S.C6.03 Mitochondrial failure in monocytes immunocompromised the NLRP3 inflammasome in human sepsis
P. Pelegrín;
Biomedical Research Institute of Murcia-Hospital Virgen de la Arrixaca, Murcia, Spain.

Sepsis is the leading cause of death in critical-care units. Systemic infection in sepsis induces the release of pro-inflammatory cytokines by monocytes, causing an uncontrolled inflammatory response damaging different tissues and organs. This inflammatory response is followed by an acute immunoparalysis due to broad defects in monocytes metabolism after the infection. In this study, we aim to characterize potential mechanisms leading to mitochondrial metabolism downregulation in monocytes and its implication in the immunoparalysis response of septic patients. We analyzed monocytes from patients with abdominal origin sepsis compared with control groups of healthy donors and patients undergoing abdominal surgery but not developing sepsis. We found that the cell surface expression of the ion channel P2X7 receptor increased in septic monocytes when compared with the control groups. Despite the increase of P2X7 receptor, in septic patients ATP failed to induce NLRP3 inflammasome activation in monocytes measured by ASC aggregation and IL-1β release. In septic patients, P2X7 receptor expression in monocytes correlated with a lack of mitochondrial membrane potential. P2X7 receptor stimulation in human monocytes from healthy individuals before LPS-priming, induced a decrease of mitochondrial membrane potential and impaired the respond of the monocytes to LPS and the engagement of the NLRP3 inflammasome. Our results suggest that during sepsis P2X7 receptor expression increases in monocytes and damage mitochondria contributing to the immunoparalysis of these patients.

S.D2 Innate lymphoid cells - a topic of debate

S.D2.01 Using super-resolution microscopy to watch immune cells kill
D. M. Davis;
University of Manchester, Manchester, United Kingdom.

Natural Killer (NK) cells can directly kill diseased cells by secretion of cytolytic granules across an immune synapse. The molecular choreography that leads to assembly of the synapse and the secretion of granules has widely been studied. However, a long-standing gap in our understanding of this process is how disassembly of the synapse occurs, allowing NK cells to dissociate from target cells. Using microscopy to visualize degranulation from individual NK cells, we found that the outcome of sequential stimulation depended upon the order in which different NK receptors were ligated. Moreover, we found that shedding of the Fc receptor CD16 increased NK cell motility and facilitated detachment of NK cells from opsonized target cells. Disassembly of the immune synapse caused by CD16 shedding aided NK cell survival and boosted serial engagement of target cells. Thus, counter-intuitively, shedding of CD16 can positively impact immune responses. In a separate line of research, using super-resolution microscopy, we have found that inhibitory Killer Ig-like receptors (KIR) encoded by different genes and alleles organise differently at the surface of primary human NK cells. KIR which are expressed at a low level at the cell surface assemble in smaller clusters than KIR which are highly expressed. Upon receptor triggering, lowly expressed receptors generate more phosphorylated Crk than highly expressed receptors. Thus, genetic variation modulates the nanoscale organisation of inhibitory KIR, which in turn impacts receptor signalling. This identifies a new way in which genetic diversity could impact immune responses.

S.D3 Novel approaches to vaccinology

S.D3.01 Viral vaccine delivery systems
S. Gilbert;
 Jenner Institute, University of Oxford, Oxford, United Kingdom.

Viral vectored vaccines are in development for many different vaccines to prevent infections, and also as therapeutic vaccines against cancer. As platform technologies they have many advantages, including reduced development timelines, ease of manufacture and the ability to employ thermostable formulations. This talk will include some recent developments in the use of adenoviral vectored vaccines against emerging pathogens, covering both preclinical and clinical data. The Jenner Institute has established a pipeline for early stage vaccine development with all activities from vaccine design, pre-clinical production and testing, GMP manufacturing and phase I clinical trials all taking place on the same campus in Oxford. This approach has facilitated the early development of multiple novel vaccines.

S.D3.03 Therapeutic monoclonal antibodies
A. Lunazvecchia;
Institute for Research in Biomedicine, Bellinzona, Switzerland.

We use cell culture-based high-throughput screens to isolate monoclonal antibodies selected for neutralizing potency and breadth. Recently, we focused on the antibody response to P. falciparum. In one study (Piiper et al, Nature 2017), we discovered that up to 10% of malaria-infected individuals produce a new type of antibodies that contain templated DNA insertions encoding the extracellular domain of LAIR1, a collagen binding inhibitory receptor encoded on chr19. These insertions are found either at the V-DJ junction or in the switch region, leading to the positioning of the LAIR1 domain on the tip of HCDR3 or in the VH-CH3 elbow. The inserted LAIR1 domain is necessary and sufficient for binding to infected erythrocytes and somatic mutations abolish collagen binding and modulate binding activity to the parasite antigens, which we identified as distinct RifinRs. Templated insertions are frequently found in memory B cells of healthy individuals, suggesting that this represents a new mechanism of antibody diversification. In another study (Tan et al Nat Med 2018), we analysed the antibody response of African individuals immunized by repeated injection of irradiated sporozoites. All antibodies isolated bound to the circumsporozoite protein (CSP) and recognized distinct epitopes. Strikingly, the most effective antibodies bound not only to the NANP-repeat region, but also to an N-terminal NPDH peptide that is not present in the RTS.S vaccine. These dual-specific antibodies were isolated from different donors and were encoded by certain VH3-30 alleles and provide relevant information for lineage-targeted vaccine design and passive immunization strategies.
S.E1.03 Spatiotemporal dynamics of CD8⁺ T cells undergoing intrahepatic priming
M. Iannacone
San Raffaele Scientific Institute, Milan, Italy.

CD8⁺ T cell responses to hepatotropic intracellular pathogens such as hepatitis B virus (HBV) range from tolerance to full differentiation into effector cells endowed with antiviral potential. However, the molecular and cellular mechanisms underlying these distinct outcomes are incompletely understood. Here, we used multiphoton intravital microscopy, RNA-seq and ATAC-seq to interrogate the motility, transcriptional and epigenetic changes of naive HBV-specific CD8⁺ T cells undergoing intrahepatic priming. We found that intrahepatic priming can lead to both effective or dysfunctional CD8⁺ T cell responses. Priming by Kupffer cells leads to differentiation into effector cells that are indistinguishable from those primed in secondary lymphoid organs; these effector cells form dense, poorly perfusable clusters that are scattered throughout the liver and are composed by largely immotile cells. By contrast, priming by hepatocytes leads to local activation, vigorous proliferation but lack of differentiation into inflammatory cytokine-producing and cytolytic effector cells; these dysfunctional cells accumulate in looser, intravascular clusters that coalesce around portal tracts and are composed by more motile cells. Transcriptome and epigenome analyses of these dysfunctional cells reveal a signature that is distinct from that of exhausted cells, including the lack of modulation of genes that are downstream of the cytokine IL-2; accordingly, CD8⁺ T cells primed by hepatocytes are refractory to anti-PD-L1 treatment but can be rescued by interleukin-2. These findings reveal the dynamic behavior of naive CD8⁺ T cells undergoing intrahepatic priming and suggest potential strategies for the therapeutic restoration of dysfunctional CD8⁺ T cells during chronic HBV infection.

S.E2.01 Human Systems Immunology - Cell by Cell
J. L. Schultz;
LIMES-Institute, University of Bonn, Bonn, Germany.

During the last three decades, immunology was characterized by work mainly in murine model systems, gene knockout technologies and a focus on single pathways or single genes. However, to understand the immune system as a system, we actually have to develop multi-science approaches interacting and collaborating with experts from other fields including genomics, cell biology, bioinformatics, and even mathematics. Furthermore, with increasing knowledge about regulatory elements that are not evolutionarily conserved between species, we need to switch to research in humans to better understand major human diseases. I will lay out and exemplify the path towards a truly human systems immunology strategy as the basis to better understand inflammatory conditions throughout the major human diseases. Single cell genomics technologies will play an important step forward to better describe and understand the role of certain immune cells in organ homeostasis, but even more so during the development and progression of major diseases. For example, by single cell RNA-sequencing, multi-color flow cytometry and functional testing we are currently mapping the human immune system in healthy and diseased lungs with highest resolution currently possible. Such approaches allow us now to define whether organ-resident immune cells or natural immigrants are the major players during immunopathologies such as asthma or chronic obstructive pulmonary disease. Furthermore, the combination with therapeutic interventions allows us even to study immune system response under perturbation conditions. Collectively, the technological revolution in genomics towards single cell resolution will greatly impact on our possibilities to directly study disease in humans.

S.E2.02 How to make sense out of big data in immunology: The single cell genomics revolution as an example
R. Zinkernagel;
University of Zurich, University Hospital, Zurich, Switzerland.

I shall try to illustrate how immunological research may be divided into 1) Surprising observations motivating experimental analysis or 2) Experiments begging for a question (P. Medawar). As long as we know little, new findings only rarely can be placed properly within a co-evolutionary context, because our methods of measurement are inadequate. Once we know almost everything any new data (also from big data) can be interpreted much more adequately. Disease and death are excellent motivators for the analysis of aetiology and rate limiting steps by using adequate methods (including big data) as a starting point to improve our understanding of immunology, immunity and evolution. Big data analysis alone is a waste of money, but as a method will help to make observation-driven analysis quicker and medically helpful, if kept within an evolutionary context.

S.E2.03 How to make sense out of big data in immunology: The single cell genomics revolution as an example
J. J. C. Neefjes;
Leiden University Medical Center, Leiden, Netherlands.

MHC class II molecules control many immune responses by presenting antigenic fragments acquired in the endosomal system to CD4⁺ T cells. Both tissue selective expression and the cell biology are complex and involve many different factors and systems. I will present how multi-dimensional screens can help placing proteins in pathways controlling MHC class II expression in an unbiased manner. This will be illustrated by a number of examples showing new pathways in control of MHC class II release in immature DC (effectively generating a mature DC phenotype), factors in control of MHC class II expression and the control of MHC class II expression in non-APC cells. In the latter case, a genetic and chemical screen was integrated that yielded both pathways and drugs in control of MHC class II expression with impact for associated diseases.
S.E4 Cell communication and signaling in the immune system

S.E4.01
Common and distinct Immune functions of exosomes and other extracellular vesicles

C. Thery, M. Tkach;
INSERM U932, Institut Curie, Paris, France.

Cells secrete into their environment different types of extracellular vesicles (EVs) that have distinct properties depending on their intracellular site of origin. Exosomes are a subtype of EVs with a mean diameter lower than 150 nm that are formed inside multivesicular compartments of the endocytic pathway. Exosomes secreted by dendritic cells (DCs) have been shown to bear functional MHC class I and class II molecules able to activate cognate T lymphocytes and induce anti-tumor immune responses. These findings motivated the use of DC-derived exosomes in cancer clinical trials, although with limited clinical effects. Other EVs also bear functional immune molecules and may thus represent alternative immunotherapy tools. In our recently published work, we have isolated different subtypes of EVs simultaneously released by live human primary DCs to characterize their protein composition, and their abilities to activate T lymphocytes. We have observed that all EVs activate T cells as efficiently, but that the resulting functionality of T cells is different. Interestingly, exosomes were not the most efficient T-cell-activating EVs. Differences in the relative levels of surface co-stimulatory proteins in the different EV subtypes can explain differences of activities. We are now analysing the common and different abilities of EVs secreted by tumor cells to induce immune responses. Our results highlight the need to determine the respective roles of exosomes and other EVs, in cancer-immune system cross-talk but also in many other patho-physiological systems, to identify the best therapeutic or diagnostic EV-based tools.

S.E4.03
Metabolic programs controlling immune cell function

T. Sparwasser;
Institute of Medical Microbiology and Hygiene, Johannes Gutenberg-University, Mainz, Germany.

Recent advances in the field of immunometabolism support the notion that essential processes in T cell biology, such as TCR-mediated activation and T helper lineage differentiation, are closely linked to changes in the cellular metabolic programs. Although the main task of the intermediate metabolism is to provide the cell with a constant supply of energy and molecular precursors for the production of biomolecules, the dynamic regulation of metabolic pathways also plays an active role in shaping T cell responses. Key metabolic processes such as glycolysis, fatty acid and mitochondrial metabolism are now recognized as crucial players in T cell activation and differentiation, and their modulation can differentially affect the development of T helper cell lineages. We only begin to understand the diverse metabolic processes that T cells engage during their life cycle from naive towards effector and memory T cells. Many milestone discoveries in this active area of research are based on the use of chemical inhibitors that have been shown to possess off-target effects, emphasizing the importance of genetic models to study immunometabolism. Following activation, T cells switch to fatty acid synthesis, demonstrating that de novo lipid synthesis actively supports T cell proliferation and differentiation. We could show previously that pharmacological or genetic ACC1 inhibition impairs T helper cell induction, with the strongest impact on Th17 development. Here we discuss the molecular mechanisms that link metabolic changes with the control of gene expression.
**JS.01 Trends in Vaccinology**

**JS.01.02**

Novel vaccines against old foes: Dengue, Zika, Ebola & Co

F. X. Heinz;
Medical University of Vienna, Center for Virology, Vienna, Austria.

Emerging viruses pose great challenges to global health and require enormous efforts for their control, as exemplified by the recent outbreaks of Ebola and Zika viruses and the continuous fight against dengue viruses, causing by far the highest number of arbovirus infections in tropical and subtropical regions worldwide. In addition to other means of outbreak control, vaccines cannot only contribute to manage acute emergencies but also provide long-term immunity to populations at risk. Probably the best example for a highly successful vaccine against an emerging virus is the live-attenuated yellow fever vaccine, which was developed ingeniously about 80 years ago in the absence of detailed knowledge of viral molecular biology, immunology and pathogenesis. Today, the armamentarium in the search for vaccines has increased impressively and led to the establishment of so-called vaccine platforms that can be readily exploited for developing vaccines against a variety of viruses, including Zika virus. These platforms include vector vaccines, recombinant immunogens produced in soluble or particulate forms, DNA and RNA vaccines, genetically engineered attenuated viruses, and chimeric viruses that can be used as live vaccines. Such chimeric replication-competent viruses are currently used and further evaluated as Ebola and dengue vaccines. Results have been encouraging, but especially in the case of dengue also provided evidence for potential negative side effects, related to an intrinsic problem of dengue pathogenesis that interferes with vaccine performance. New technologies like structure-based vaccine design may be starting points for developing more effective immunogens.

**JS.01.03**

A new generation of vaccines: for each target group its own adjuvant?

E. C. Lavellie;
Trinity College Dublin, Dublin, Ireland.

The effectiveness of vaccines and their capacity to promote and direct adaptive immunity depends on the induction of specific types of innate immune responses. As we increasingly adopt subunit vaccines which depend on adjuvants for their immunostimulatory potential, there is scope to refine vaccine formulations for specific conditions and defined target groups. Innovative adjuvant approaches can allow vaccines to be targeted at specific groups, for example the elderly or neonates. However, detailed knowledge of the nature of immune regulation in such groups and of the expression of specific innate immune sensors is required to facilitate rational vaccine design. Innate immune factors such as type 1 interferons and inflammasomes can play key roles in promoting adaptive immunity and identifying how adjuvants can regulate these and other such pathways which direct adaptive immunity can provide valuable targets for vaccine design. Adjuvants can also facilitate a move from injectable to oral vaccines which are attractive for the many enteric infections we are faced with. In conclusion, advances in our understanding of how innate immunity impacts on adaptive responses and in the design and formulation of adjuvants allows greater potential for a targeted approach to vaccination in future.

**JS.02 Systems Immunology for stratifying patients with autoimmune diseases**

**JS.02.03**

The Stratification of Lupus

M. Alarcón Riquelme;
Center for Genomics and Oncological Research (GSEVO), Granada, Spain.

**Objectives:** The highly heterogeneous clinical presentation of lupus is characterized by the unpredictable appearance of flares of disease activity and important organ damage. Attempts to stratify lupus patients have been limited to clinical information, leading to unsuccessful clinical trials and controversial research results. Our aim was to develop and validate a robust method to stratify patients with lupus according to longitudinal disease activity and whole-genome gene expression data in order to establish subgroups of patients who share disease progression mechanisms. **Methods:** We applied a clustering-based approach to stratify SLE patients based on the correlation between disease activity scores and longitudinal gene expression information. Clustering robustness was evaluated by bootstrapping and the clusters were characterized in terms of clinical and functional features. **Results:** Using two independent sets of patients, one pediatric and another adult, our results show a clear partition into three different disease clusters not influenced by treatment, race or other source of bias. Two of the clusters differentiate into a neutrophil correlated disease group and a lymphocyte correlated disease group, while the third that correlated to a lesser extent with neutrophils, was functionally more heterogeneous. The neutrophil-driven clusters were associated with increased development towards proliferative nephritis. **Conclusions:** We found three subgroups of patients that show different mechanisms of disease progression and are clinically differentiated. Our results have important implications for treatment options, the design of clinical trials, the etiology of the disease, and the prediction of severe glomerulonephritis.

**JS.03 Antigen Presentation in Health and Disease**

**JS.03.02**

Antigen processing and presentation in cancer immunosurveillance

R. Binder;
University of Pittsburgh, Pittsburgh, PA, United States.

Binder, Robert

University of Pittsburgh, Pittsburgh PA

The immune system recognizes aberrant cells and eliminates them prior to emergence of nascent tumors. This prevents progression of many malignancies. In the absence of such immunity in mice or humans, multiple and frequent tumors are generated. Current immunosurveillance model involves the priming of T cell and NK cell immunity. The gap in knowledge in this model is raised in two questions: (1) What is the molecular mechanism for cross-priming T cell responses in the context of the negligible amount of antigen available at the early stages of nascent tumor development? (2) What is the stimuli for co-stimulation of T cell priming and activation of NK cells. Both of these questions are unanswered. Our work has demonstrated that tumor-derived heat shock proteins (HSPs), introduced during vaccination, are super-efficient at cross-presentation of limited amounts of their chaperoned tumor (peptide) antigen. HSPs are also capable of initiating signals for co-stimulation. Both events require the HSP receptor, CD91, expressed on

**JS.03.03**

Generating peptide-MHC ligands for immune surveillance of foreign and self

N. Shastry1, C. Park2, J. Guan1, T. Ding1, F. Gonzalez2;

1University of California, Berkeley, CA, United States, 2Johns Hopkins University School of Medicine, Baltimore, MD, United States.

The normal peptide repertoire presented by classical and non-classical MHC class I molecules is regulated by ERAAP, the endoplasmic reticulum aminopeptidase associated with antigen processing. Loss of ERAAP's peptide trimming function in cells causes dramatic changes in the peptide repertoire. The changes in the peptide repertoire enhance the immunogenicity of ERAAP-deficient cells and elicit potent immune responses in otherwise syngeneic wild-type mice. Because changes in ERAAP activity can cause abnormal immune responses, normal ERAAP function is monitored by an unusual subset of semi-invariant CD8+ T cells. These T cells recognize the QFL ligand that consists of a conserved peptide presented by the non-classical Qa-1/MHC Ib molecule displayed only on surface of ERAAP-deficient cells. We show that these QFL-specific CD8+ T cells (QFL-T cells) bear unique and semi-invariant αβ TCRs. Genetic manipulation of the expression of the self-QFL ligand and functional characteristics of QFL-T cells shows that in addition to monitoring ERAAP function, QFL-T cells may also regulate metabolic activity.

**Novel vaccines against old foes: Dengue, Zika, Ebola & Co**

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Efferocytosis induces a novel SLC program to promote glucose uptake and lactate release


On a daily basis, we turnover billions of apoptotic cells that are removed by professional and non-professional phagocytes. While characterizing the transcriptional program of phagocytes, we discovered a novel solute carrier family (SLC) gene signature (33 SLC members) that is specifically modified during engulfment of apoptotic cells (efferocytosis) but not during antibody-mediated phagocytosis. We have recently observed fundamental differences in the phenotype and function of monocytes stimulated via either TLR7 or TLR8, in the context of RNA virus infections, and specifically, in terms of type I IFN responses and effector cytokines they produce, as well as general differences in cell surface markers. We have defined the molecular mediators that are responsible for these differences in phenotype by performing ex vivo experiments with human monocytes isolated from blood and we have shown the relevance of these data in common RNA virus infections, demonstrating that TLR7 and/or TLR8 stimulation by RNA virus infections of human monocytes account for much of the phenotype the cells acquire upon virus interaction.

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JS.08 Cytometry building bridges

JS.08.01 Cytometry of aging of the immune system
A. Cossarizza; University of Modena and Reggio Emilia, Modena, Italy.
In most developed and rich countries, human life span has dramatically increased because of advances in preventing, delaying, or curing several pathologies. In all mammals, the aging process is characterized by profound changes in immunological responses that can be identified either analyzing the phenotype of lymphocytes (typically, collected from peripheral blood in the case of donors) or their functionality. For these purposes, several techniques have been developed in the last decades, but with no doubts flow cytometry has assumed the main role, due to the possibility to analyze several parameter (at present, up to 27 by using fluorescent dyes, more than 40 by mass cytometry) at the single cell level. Thus, a very fine characterization of age-related changes is occurring, and, for example, researchers are clarifying the importance of exhaustion markers among different subsets of T cells as predictors of morbidity and mortality, building a new immune risk profile. Not only cytometry is now allowing to identify such a huge amount of parameters, but also to identify rare cells, i.e. those present in a percentage that is much less than 1 in one thousand, like those specific for a given antigen. So, for example, the identification of responding cells is crucial for a better understanding of the effect of vaccines in the elderly. The talk will present some recent advancement in the field of immunology of aging that have been obtained by studies at the single cell level.

JS.08.03 Standardizing cytometry of primary immunodeficiencies
T. Kalia; Charles University, Prague, Czech Republic.
EuroFlow consortium has developed and validated a set of 8-color flow cytometry panels for the initial evaluation of patients with the clinical suspicion of a Primary Immunodeficiency (PID). We have analyzed 96 PID patients and additional 21 severe PID diagnosed until the age of 2 years. I will aim to illustrate following objectives:
- How and why to do standardized measurements in an international collaboration
- Investigation of lymphocyte subsets (incl. their maturation status) in one tube can be used to assess abnormalities in primary immunodeficiency
- Approach to comprehensive visualization of results
- Search for hallmarks
- Present an example of diagnostic utility of the two tube test for sever PID (SCID, Omenn syndrome, CID and other PIDs with molecular lesion at the age 0-2 years)

In summary, out of the 96 PID patients we failed to uncover lymphocytes’ subsets abnormality only in 2 patients with Chronic Granulomatous Disease and 4 patients with complement deficiencies (in line with literature), but we were able to find at least one abnormality in all remaining patients with PID. Furthermore the T-cell directed tube revealed accurately all SCID and Omenn syndrome patients even in cases with maternal engraftment. This is the first approach to a standardized PID diagnostic test that should reveal all alterations in patient lymphocytes’ compartment without a correct a priori assumption about the PID nature (a universal test).

Acknowledgement: Supported by EuroFlow and a grant 15-28541A and LG1604.

JS.09 The gut microbiota and the IgA antibody production in health and disease

JS.09.03 The regulatory microenvironment in Peyer’s patches leading to synchronized gut IgA responses
N. Lycke1, M. Bemark1, K. Kombari1, A. Strömberg1, Z. Shulman2.
1Gothenburg, Sweden; 2University of Gothenburg, Gothenburg, Sweden. 3The Weizmann Institute of Science, Rehovot, Israel.
The majority of activated B cells differentiate into IgA plasma cells at mucosal sites, with the gut being the largest producer of immunoglobulin in the body. Secretory IgA antibodies have numerous critical functions of which protection against infections and the role for establishing a healthy microbiota appear most important. Expanding our knowledge of the regulation of IgA B cell responses and how effective mucosal vaccines can be designed are of critical importance. I will discuss recent developments in this field that shed light on the uniqueness and complexity of the gut mucosal IgA inductive site, the Peyer’s patches. In particular, I will describe a novel B cell dependent pathway for bringing luminal antigens from the M cell to the germinal centers in the Peyer’s patches. In addition, single cell RNAseq data on the composition of antigen-specific B cells in the Peyer’s patches following oral immunizations will be presented.

JS.10 Innate host pathogen interactions

JS.10.01 Protective role of Mincle in Gram-positive bacterial infection
S. Iwasaki; Osaka University, Osaka, Japan.
C-type lectin receptors (CLRs) comprise a large family of proteins that share a common structural motif and are involved in various immune responses. Among them, ITAM-coupled CLRs are recently identified as pattern recognition receptors (PRRs) for pathogens. Mincle (Macrophage-inducible C-type lectin) is an FcγRI-coupled activating receptor that recognizes mycobacterial glycolipid, trehalose dimycolate, to promote protective immunity against mycobacteria. Recently, we found that Mincle also recognizes Gram-positive bacterial pathogen, Group A Streptococcus (GAS). GAS causes fatal invasive infections and thus called “flesh-eating bacteria”; however, the mechanism by which our immunity reacts to this pathogen is not well understood. Within GAS components, we purified and identified unique glycolipids as Mincle ligands. Upon invasive GAS infection, Mincle-deficient mice exhibited impaired cytokine production of pro-inflammatory cytokines, severe bacteremia and rapid lethality. These results indicate that Mincle plays a central role in protective immunity against acute GAS infection.

JS.10.02 LILR family receptors in host pathogen interactions
H. Arase1; 1Research Institute for Microbial Diseases, Osaka University, Suita, Japan, 2Immunoology Frontier Research Center, Osaka University, Suita, Japan.
Immune cells express various kinds of paired receptors that consist of inhibitory and activating receptors. Although their extracellular domains are highly homologous between inhibitory and activating receptors, inhibitory receptors possessing ITAM at cytoplasmic domain downregulate activation of immune cells, whereas activating receptors deliver activating signals via ITAM positive adaptor molecules like DAP12. LILR family receptors are one of representative paired receptors mainly expressed on innate immune cells. From the analysis of LILR family receptors, we found that inhibitory LILRB1 is used by Plasmodium falciparum for immune evasion. Furthermore, immune evasion of Plasmodium falciparum from LILRB1 was associated with severe malaria (Nature 2017). On the other hand, we have found that LILRA2, one of activating LILR family receptors, specifically recognizes immunoglobulin cleaved by bacterial protease whereas LILRA2 does not recognize normal immunoglobulin. This suggested that activating LILRA2 plays an important role in host defense by sensing immunoglobulin abnormalities (Nature Microbiology 2016). These studies suggest that LILR family receptors play an important role in host-pathogen interaction.

JS.10.03 IL2C and type 2 immune diseases
K. Moro; RIKEN IMS, Yokohama, Japan.
Recent studies have revealed new types of lymphocytes functioning in innate immune responses that are collectively called innate lymphoid cells (ILCs). Unlike T and B lymphocytes, ILCs lack Rag-dependent antigen-specific receptors and are activated by cytokines produced by other innate immune cells or epithelial cells. ILCs have been divided into 3 groups based on their cytokine production profiles; group 1 ILC including NK cells and ILC1 produce IFNγ, group 2 ILC (ILC2) including natural helper cells, nuocytes and innate helper type 2 cells produce type 2 cytokines such as IL-5, IL-6 and IL-13, and group 3 ILC including lymphoid tissue inducer (LTI) cells and ILC3s produce IL-17 and IL-22. ILCs play important roles in protection against various invading microbes including multicellular parasites, and in the maintenance of homeostasis and repair of epithelial layers. ILC2 produce a large amount of IL-5 and IL-13 in response to IL-25 or IL-33, and induce eosinophilia and goblet cell, both of which act to protect against helminth infection and exacerbation of allergy. Since we discovered ILC2 in 2010, many other research groups have joined this research field and identified new immune responses that are regulated by ILC2. In particular, the importance of ILC2 in allergic diseases has received a fair amount of attention and new evidence indicates that allergic disorders occur not only from allergen-specific pathways but are also induced by allergen non-specific pathways due to ILC2 activation.
EDUCATIONAL SESSIONS
EDU.01 Systems Biology for Immunology: Help with the Complexity

EDU.01.03 Systems Immunology: Making sense of the Yins and Yangs
H. V. Westerhoff;
AIMMS and SILS and MCI5B, Amsterdam and Manchester, Netherlands.

Thousands of molecules are discussed at this conference. The good news is that most can now be measured quantitatively. Immunology must thereby be the most challenging science for which most of the building blocks of complexity can be measured. How can they be understood however? Will the Biology that draws diagrams and arrows, suffice? The interactions between immunological factors and phenotype or disease appear to be determined by multiple networks that work positively and multiple that work negatively. For what we want, i.e. to understand and then cure, the usual schemes with plusses and minuses won’t do. In an analogy with Deep Sequencing, this conference brings ‘Deep Immunology’ to the fore, i.e. a strategy that collects ‘all’ data and then puts these into a ‘machine’ that helps understand what they imply. Such a ‘machine’ is a new type of mathematical model, i.e. a ‘watchmaker model’, which is able to assimilate all mechanisms into a coherent and predictive whole; a whole that may not be greater than the sum of its parts, but actually smaller (simpler), and certainly different from that sum. This presentation will present one such model, which deals with multiple concatenated regulatory networks of various signs and strengths that affect the innate immune response. It will show how the model gives a handle on understanding the dichotomy between acute and chronic inflammation and what this has to do with supplementation of stem cells in immune therapies. And it will show how immunologists can now use this Deep Immunology.

EDU.02 The Utility of Therapies in Immunology

EDU.02.02 The “two-niche” theory of T cell Memory
F. Di Raso;
Institute of molecular biology and pathology, Italian National Research Council (CNR), Rome, Italy.

The concept is emerging that the bone marrow (BM) sustains life-long persistence of memory T cells, as it does for long-lived plasma cells. The majority of BM memory CD8 T cells are recirculating cells in constant exchange with blood, although some may permanently reside in the BM, thus resembling tissue-resident memory CD8 T cells of extra-lymphoid organs (e.g. gut, skin, etc.). Indeed, it has been suggested that the BM provides “niches” for memory CD8 T cell self-renewal, thus controlling the maintenance of memory CD8 T cell number in the body. However, many gaps remain unfilled, and in particular how memory CD8 T cells are steadily maintained as seemingly quiescent cells, and yet are poised to promptly generate a huge progeny of effector CD8 T cells upon secondary response. In an active debate on the regulation of memory T cell quiescence, I have built upon my own and others’ data to propose a novel hypothesis: namely, that the BM offers two types of niches to promote T cells’ driving self-renewal, the other supporting quiescence, thus echoing the duality of niches for hematopoietic stem cells. While self-renewal would stably preserve memory T cell numbers, maintenance in a quiescent state would preserve a capacity to promptly mount secondary responses. Hence, testing this hypothesis is key to considering how CD8 T cell responses to discrete challenges may be beneficially manipulated.

EDU.02.03 The Discontinuity Theory of Immunity
E. Vivier1; T. Pradeu2;
1Alis Marseille Univ; CNRS, INSERM, APHP, CIMAL & Innate Pharma, Marseille, France, 2Unité d’Immunologie (ImunoConcEpT, UMR5164), Université de Bordeaux, France.

Similar to many other biological systems, the immune system can be seen as a change-detection system. According to the discontinuity theory of immunity, the immune system responds to sudden changes in antigenic stimulation and is rendered tolerant by slow or continuous stimulation. The immune system can adapt to these slow or long-lasting modifications in the host, which it then treats as a new reference point. This basic principle, which is supported by recent data on immune checkpoints in viral infections, cancers, and allergies, can be seen as a unifying framework for diverse immune responses. The mechanisms underlying the recognition of patterns, the absence of a pattern, tissue damage, and functional modifications have been considered separately, but the discontinuity theory of immunity explains these mechanisms as instances of the more general rule that immune systems have been selected by evolution to respond to sudden modifications within the host.

EDU.02.04 The Equilibrium Model of Immunity
G. Eberl;
Institut Pasteur, Paris, France.

The classical model of immunity states that the immune system reacts to pathogens and injury and restores homeostasis. Indeed, a century of research has uncovered the means and mechanisms by which the immune system recognizes danger and regulates its own activity. However, this classical model does not fully explain complex phenomena, such as tolerance, allergy, the increased prevalence of inflammatory pathologies in industrialized nations and immunity to multiple infections. I propose a model of immunity that is based on equilibrium, in which the healthy immune system is always active and is in a state of dynamic equilibrium between antagonistic types of response. This equilibrium is regulated both by the internal milieu and by the microbial environment. As a result, alteration of the internal milieu or microbial environment leads to immune disequilibrium, which determines tolerance, protective immunity and inflammatory pathology.

EDU.03 Immunology of extracellular vesicles

EDU.03.01 Extracellular vesicles in acute and chronic Inflammation
E. I. Buzsá;
Semmelweis University, Budapest, Hungary.

Extracellular vesicles are phospholipid membrane enclosed structures released by cells in an evolutionarily conserved manner. These subcellular structures are secreted even under steady state conditions as part of the homeostatic cell-cell communication. Importantly, upon cell activation, induction of cellular stress or different types of cell death, both the number and the molecular composition of extracellular vesicles are altered. In innate and adaptive immune reactions, extracellular vesicles convey intercellular messages by delivering their molecular cargo (proteins, lipids, nucleic RNA and DNA and metabolites). Extracellular vesicles are important carriers of danger signals. There are numerous examples showing that PAMPs and DAMPs are associated with extracellular vesicles and are recognized by the innate immune system. Furthermore, by carrying a wide variety of cytokines and surface-associated tissue degrading enzymes, extracellular vesicles are important players in both acute and chronic inflammation. They have the ability to stimulate antigen-specific T cells, and they participate in cross-dressing and cross-presentation of APCs. The role of extracellular nuclear molecules in the pathogenesis of autoimmune diseases is increasingly recognized. Rapidly growing number of data supports that extracellular vesicles are complex packages of autoantigens which often represent the focus of autoimmune reactions. Finally, they are not only promising biomarkers of diseases but also have significant therapeutic potential in diseases with immune pathomechanism.

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EDU.03.02
Isolation and detection of extracellular vesicles

R. Nieuwland;
Amsterdam UMC, Amsterdam, Netherlands.

Human body fluids contain cell-derived membrane-enclosed vesicles. The cellular origin, concentration, composition and function of vesicles in body fluids differ in health and disease. Therefore it is not surprising that the scientific and clinical interest in EVs is growing exponentially because vesicles potentially behold the promise of an entirely new set of biomarkers.

Body fluids contain multiple types of vesicles, which are involved in intercellular communication, cellular waste management, and host defence (inflammation, coagulation). Because the types of vesicles are difficult to distinguish from each biochemically (composition) or biophysically (size, density), the umbrella term “extracellular vesicles” was introduced by the International Society of Extracellular Vesicles (ISEV).

Most EVs in body fluids are extremely small and spherical, and the bulk of EVs have a diameter of less than 200-300 nm. Blood, which is the most commonly studied body fluid for biomarker research, contains not only EVs but also high concentrations of soluble proteins and lipoprotein particles / chylomicrons which overlap in size and density with EVs. Consequently, the isolation, detection and biochemical characterization of EVs is not trivial, and only recently several major methodological pitfalls and hurdles have been overcome.

In this presentation, an overview will be given about the progress that has been made regarding isolation of EVs, and the detection of single EVs by flow cytometry.

EDU.03.03
Extracellular vesicles in immunity

S. Gabrielsson;
Karolinska Institutet, Dept of Medicine, Stockholm, Sweden.

Exosomes from antigen presenting cells are interesting as potential cancer immunotherapy vehicles due to their capacity to stimulate tumor-specific activity in mice. However, clinical trials using peptide-loaded autologous exosomes showed only moderate T cell responses, suggesting a need for optimization of exosome-based therapy. We are using dendritic cell derived exosomes to induce antigen-specific immune responses with the aim to cure cancer. We have seen that exosomes need to carry whole protein, and not only peptide/MHC complexes, to induce a strong T cell response in vivo. We showed that the reason for this was that B-cells needed to be activated to induce a strong T cell response to exosomes. This has led us to test allogeneic exosomes in a B16 melanoma model, and our results demonstrate that allogeneic exosomes are as efficient in inducing immune responses as syngeneic exosomes. This greatly increases the feasibility of exosome-based immunotherapy. Currently we are working on different ways to further boost immunogenicity of exosomes and data from these studies will be presented.
EFIS PRESIDENT’S SYMPOSIUM
Tissue-resident memory T-cells (Trm) form populations of memory T-cells in barrier tissues including the epithelial compartments of lungs, skin, and gut. Trm are emerging as one of the most potent immune weapons against reinfection, prompting great interest in the development of therapeutic strategies for their elicitation. We have previously identified Hobit as a central regulator of Trm differentiation in mice. We found that the transcription factor was universally expressed in Trm populations of the skin, gut and liver, but not in circulating memory populations. Hobit together with related Blimp-1 was essential for the formation of Trm through suppression of the expression of tissue exit receptors such as S1PR1 and CCR7. Therefore, our findings indicate that Hobit and Blimp-1 mediate a universal program of tissue-residency. The restricted expression pattern of Hobit within resident lymphocytes is a characteristic that we deemed highly valuable for the development of novel tools to study Trm. Therefore, we generated a transgenic mouse model that contained a “knock-in” within the Hobit locus of an “all-in-one” construct of the tdTomato reporter, CRE recombinase and the diphtheria toxin receptor (DTR). We confirmed that expression of tdTomato and functional activity of the CRE recombinase and the DTR was nearly uniformly present and largely restricted to Trm in the transgenic mice. These findings suggest that we have established a novel model system to address Trm development in vivo. We aim to resolve the development and the potential of Trm through the further characterization of T-cell differentiation in Hobit reporter mice.
LATE BREAKING HOT TOPICS
HT.04 Late Breaking Hot Topic 4

HT.04.01
Smoking induces recruitment of monocytes into the alveolar space and contributes to accelerated growth of Mycobacterium tuberculosis

B. Corlet; J. L. Cho; D. G. Roy; A. S. Divakaruni; T. N. Tarasenko; A. N. Murphy; D. G. Roy; R. G. Jones; T. Sparwasser

Summary: Smoking is associated with a higher total lung bacterial burden, suggesting that monocyte-derived macrophages may be more permissive to Mycobacterium tuberculosis (Mtb) growth relative to resident macrophages in the lung. We recruited healthy current- and never-smokers for collection of bronchoalveolar lavage (BAL). Monocytes, BAL macrophages or monocyte-derived macrophages (MDMs) were analyzed using flow cytometry, and infected with Mtb in vitro and bacterial growth monitored over time by CFU. We examined numbers of macrophages, T cells and granulocytes in BAL and found a significant increase in a population of small macrophages in BAL from smokers compared to non-smokers. Half of the small macrophages expressed surface CD93, a marker which distinguished circulating monocytes from large alveolar macrophages and suggests that these small macrophages are derived from newly recruited monocytes. BAL fluid from smokers recruited blood monocytes in vitro and significantly higher concentrations of the chemokine CCL11 in BAL fluid correlated with the number of CD93+ small macrophages in BAL. Virulent Mtb induced a hyper-inflammatory response in human BAL monocytes with significantly higher intracellular growth compared to MDMs or large alveolar macrophages in vitro. In conclusion, our data indicate that smoking leads to higher numbers of total BAL macrophages due to CCL11 mediated recruitment of circulating CD93+ monocytes into the alveolar space. Importantly, monocytes were highly susceptible to Mtb intracellular growth, suggesting that extensive monocyte infiltration plays a significant role in smoking associated risk for active tuberculosis.

HT.05 Late Breaking Hot Topic 5

HT.05.01
Quantitative shotgun proteo-/transcriptomics shows how human regulatory T cells protect their identity

D. Amsen

Summary: Inflammation is both a requirement and a challenge for regulatory T cell (Treg) function. Inflammatory cues direct Tregs to inflamed sites where they limit tissue destruction and promote repair. Under pathological conditions, inflammation can however coerce Tregs into assuming functions normally performed by conventional T cells (Tconvs), such as production of pro-inflammatory cytokines. Here, we have studied how Tregs generally protect their identity from such destabilization. For this, we first established a molecular definition of Treg identity by performing whole cell shotgun proteomics and transcriptomics on multiple human populations of Tregs and Tconvs. We found that proteome and transcriptome compositions markedly differ from one another, but are complementary, underscoring the importance of analysis at both levels. Core and subset-specific Treg signatures reveal that these cells have specific adaptations in cytokine-, TCR- and costimulatory receptor signaling pathways. We show that strategic deficiencies in individual pathways allow inflammatory cytokines to mobilize select functions in Tregs (such as expression of transcription factors and homing receptors) without compromising Treg identity. Genetic alterations in individual signature molecules however suffice to allow conversion of Tregs into Tconvs by inflammatory cytokines. Our results therefore identify molecular nodes that determine the unique response characteristics of Tregs to inflammation.

HT.06 Late Breaking Hot Topic 6

HT.06.01
A cautionary note for using pharmacological inhibitors challenges the role of long-chain fatty acid oxidation on immune cells

L. Berod; B. Raudf; D. G. Roy; A. S. Divakaruni; T. N. Tarasenko; R. Franke; M. Bröndstrup; A. N. Murphy; P. J. McGuire; R. G. Jones; T. Sparwasser

Summary: Long-chain fatty acid oxidation (LC-FAO) has been suggested to play an important role supporting CD4+Foxp3+ regulatory T cells as well as CD8+ memory T cell differentiation and survival. Similarly, LC-FAO has been associated with IL-4-driven macrophage polarization towards the alternative M2 phenotype. However, previous research leading to these conclusions is based on the pharmacological inhibition of carnitine palmitoyltransferase-1, the rate-limiting enzyme for LC-FAO, with high concentrations of the drug etomoxir. Using genetic mouse models to target Cpt1a on specific immune cell populations, we dissected the role of LC-FAO in primary, memory, and regulatory T cell responses. Challenging previous concepts, we here show that LC-FAO and Cpt1a are largely dispensable for effector, memory, or regulatory T cell formation, and that the effects of high dose etomoxir on immune cell differentiation and function are independent of Cpt1a expression. Together our data argue that metabolic pathways other than LC-FAO fuel CD8+ memory or Treg differentiation and suggest off-target effects of etomoxir on mitochondrial respiration.
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BRIGHT SPARKS WORKSHOPS

BS.A.01 Bright Sparks A

**BS.A.01.01** Developmental origin of osteoclasts and their functional maintenance by nuclear chimerism

*E. Mess*,1,2,3 C. E. Jacome-Galarza*,1,2,3 G. I. Percini*,1,2,3 T. Lazaro*,1,2,3 J. Eitter*,1,2,3 M. Rauer*,1,2,3 L. Crozet*,1,2,3 M. Bohm*,1,2,3 C. Waskow*,1,2,3 F. Geissmann*;
1Life & Medical Sciences Institute (LIMES), Bonn, Germany, 2Maximilianeum, New York, United States, 3Institute for Immunology, Dresden, Germany, 4Leibniz-Institute on Aging-Fritz-Lipmann-Institute, Jena, Germany

Osteoclasts are multicellular macrophages that continuously remodel the bone marrow hematopoietic niche. Excess osteoclast activity contributes to bone loss and osteoporosis, while decreased activity leads to osteopetrosis and bone marrow failure. Osteoporosis can be partially treated by bone marrow transplantation in human and mice, which is in accordance with in vitro studies suggesting that osteoclasts develop by fusion of hematopoietic stem cell (HSC)-derived monocytic precursors. However, the developmental origin and lifespan of osteoclasts, and the mechanisms that ensure their maintenance and function in vivo remain largely unexplored. Here we report that osteoclasts are long-lived cells that originate from erythro-myeloid progenitors (EMP) in the yolk sac. Using genetic fate-mapping and knockout models we show that EMP-derived osteoclasts colonize ossification centers during embryogenesis and are required for normal bone development and teeth eruption. However, after birth osteoclasts depend on HSC-derived cells. Parabiosis and transfer experiments indicate that an iterative fusion of osteoclasts with circulating monocytes sustains their function is thus indispensable for the maintenance of the bone marrow niche during aging. Altogether, our results identify a dual origin of osteoclasts in vivo, and nuclear chimerism as a mechanism that enables long-term maintenance and function of these osteoclasts, and thereby suggest new strategies to modulate osteoclast activity.

**BS.A.01.02** Uncovering the regulatory T cell transcriptional signature in the human thymus

*M. Ângelo-Dias*,1 A. Godinho-Santos,1 Y. Tokunaga,1 A. Serra-Caetana,1 H. Nunes-Cabaço,1 A. E. Sousa,1 A. A. Raposo1;
1Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.

Thymic committed regulatory CD4+ T cells (Treg) are essential to maintain self-tolerance and immune homeostasis, yet there are no genome-wide data defining their expression profile in the human thymus. Although FoxP3 is known as a master regulator of Treg differentiation and function, many other factors remain to be uncovered. To this purpose, we sorted mature CD4+ single-positive thymic Tregs (1Treg) and their conventional counterparts (1Tconv) based on the expression of CD27, CD25, and CD127, from three human thymuses collected during routine corrective pediatric cardiac surgery, and generated their respective expression profiles by RNA-seq.

Our comparative transcriptomic analysis identifies ID144 differentially regulated genes significantly differentially expressed between 1Treg and 1Tconv subsets. We confirm the prominent expression of Treg associated FoxP3 (1Treg, IL2RA, CTLA4, LIRIC2, IKZF2, IKZF4) in 1Treg relative to 1Tconv. Of note, 45 amongst the 648 Treg highly-expressed genes encode for known transcription factors (TFs) including those involved in T cell activation and differentiation (RORα, AHR, TBK21, STAT4), and NF-kb pathway (NFκB2, RELB, and REL). To identify novel factors and pathways, we selected 196 transcripts uniquely expressed in 1Treg and not expressed in 1Tconv. Remarkably, we uncovered groups of genes with a strong statistical association with the regulation of cell migration (FN1, CCL2, LMNA, LAMA2, ICAM1, CXCR3), cytokine pathways (CCR8, IRF5, TNFRSF8, IL12RB2, IL1RL1, EB13), and ion homeostasis (RYR1, ACTN2, HMOX1, CHRNA6, TMPRSS6, CAV1, KCNS3, CASQ2).

Our data open several new lines of research to further clarify pathways of Treg lineage commitment in the thymus, and determinants of the human Treg signature.

**BS.A.01.03** Human Tbet+ B cells: induction and effector functions from a multiple sclerosis perspective

*J. van Langeloo*,1 R. Iriartes*,1 M. Jansen*,1 I. de Groot,1 L. Kalden,1 A. K. Wierenga-Wolff,1 S. Koetsier,1 T. A. Siermappa,1 H. E. de Vruijs,1 P. Unger,1 M. S. van Ham,1 R. Q. Hintzen*,1 M. M. Luijn1;
1Dept. Immunology, MS Center Erasus MS, Erasmus MC, Rotterdam, Netherlands, 2Dept. Neurology, MS Center Erazus MS, Erasmus MC, Rotterdam, Netherlands, 3Dept. Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, Netherlands, 4Dept. Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.

In multiple sclerosis (MS), peripheral B-cell tolerance checkpoints are defective and meningeal B-cell follicle-like structures are present in the central nervous system (CNS). Both these critical events link to the function of IFNγ- induced autoreactive T-bet+ B cells in mice. However, the exact B-cell subpopulations and mechanisms contributing to disease activity in MS patients are underevaluated. Here, we found increased STAT1 phosphorylation in IFNγ-triggered human B cells carrying a recently identified coding MS risk variant, IFNGR2. In 132 CD40L/IL-21 based cultures, B cells from MS patients revealed higher T-bet and enhanced ASC differentiation in the presence of IFNγ compared to matched controls. When CpG GD40 was added, T-bet and CXCR5 were further upregulated and caused enhanced switching to IgG1. Ex vivo FACS analysis of B cells showed that CXCR5(IgG)s and not naive or non-class-switched cells were reduced in untreated MS versus control blood and were enriched in paired cell suspensions from MS brain tissue, meninges and cerebrospinal fluid. CXCR5 and not CXCR3, was selectively upregulated on accumulating IgG1 B cells in natalizumab-treated MS blood (pre- versus 1-year post-Tx). Correspondingly, the abundance of CXCR3 on IgG1 B cells led to superior transmigration across human brain endothelial monolayers in vitro. Finally, CXCR5+/CXCR3- and IgG1/CXCR3+/CXCR5- B-cell frequencies correlated to those of IFNγ+ Th17.1 and not Th17 cells in MS blood. This work demonstrates that T-bet is synergistically upregulated by IFNGR and TLR9 in B cells, probably underlying preferential CXCR3-mediated recruitment to the CNS and enhanced IgG1 responses to local antigens in MS.

**BS.A.01.04** Extracellular Vesicles derived from licensed Mesenchymal Stem Cells: a tunable approach to regulate inflammatory angiogenesis

*R. Angioni*,1,2 S. Herkenne*,1,2 R. Sanchez-Rodriguez*,1,2 B. Ceñal1,2,3,4, B. Giron*,1,2,3,4, A. Viola1,2,3,4;
1Padova University, Department of Biomedical Sciences, Via Ugo Bassi, 51, 35121 Padova, Italy, 2Padova, Padova, Italy, 3VIMM- Venetian Institute of Medical Science, Padova, Italy, 4Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy

Angiogenesis is the process that leads to the formation of new blood vessels from a pre-existing vascular network, playing a key role in many physiological and pathological processes. Consequently, targeting angiogenesis represents a very interesting therapeutic approach. We have already shown that mesenchymal stem cells (MSCs) stimulated with pro-inflammatory cytokines (st-MSCs) block angiogenesis through the release of soluble factors. Thus, we highlighted the endothelium as a novel target during the MSC immunosuppressive effect. However, the therapeutic employment of MSCs to control inflammation is far to be clinically translated. The development of a cell-free therapeutic approach could represent a better cost-effective and safer procedure. Here, we demonstrate that extracellular vesicles derived from stimulated MSC conditioned medium (EV stMSC-CM), but not from their unstimulated counterparts (EV unstMSC-CM), affect angiogenesis, thus recapitulating the MSC effect. EV stMSC-CM, expressing high levels of the ecto-5'-nucleotidase CD73, generate extracellular adenosine. We demonstrated that the EV stMSC-CM generated adenosine inhibits the endothelial cell migration, by inducing an excessive intracellular ROS accumulation, both in vivo and in vitro. This work reveals that EVs derived from st-MSCs display anti-angiogenic properties and could be exploited for cell-free therapeutic strategies. Additionally, they pave the way for a better understanding of the MSC physiological role in vivo.

**BS.A.01.05** Lymph node stromal cell function upon immune activation is modulated by dynamics between podoplanin and its binding partners on the plasma membrane

*C. M. de Wind*,1 A. C. Benjamin1, L. Millward,1 V. G. Martinez,1 S. E. Acton;
1MRC Laboratory for Molecular Cell Biology, London, United Kingdom.

Lymph node explant is a pivotal process during activation of an immune response, and is controlled by contractility through the fibroblastic reticular cell (FRC) network. Interactions between CLEC-2 expressed on dendritic cells (DCs) and podoplanin on FRCs results in rapid actomyosin contractility and subsequent elongation of FRCs allowing lymph node explant. Directly downstream, this interaction results in decreased membrane binding and phosphorylation of ezrin-radixin-moesin (gERM) proteins. We sought to understand the molecular mechanisms controlling podoplanin activity.

Podoplanin remains at the plasma membrane even when inhibited by CLEC-2. We hypothesized that podoplanin function is regulated by specific binding partners in different membrane compartments. Protein clustering within membranes is tightly controlled by specialized membrane structures, including lipid rafts and tetraspanin-enriched microdomains. Upon CLEC-2 binding, podoplanin preferentially clusters into cholesterol-rich membrane domains, containing CD44, a known podoplanin binding partner. We show a link between podoplanin and CD44 expression in FRCs. Knockdown of podoplanin coincides with decreased CD44 expression, and in contrast, CLEC-2 stimulation of FRCs increases CD44 expression. RNAseq analysis identified tetraspanins CD9 and CD82 as potential interaction partners of podoplanin. Similarly to CD44, expression of both CD9 and CD82 was altered by contractility, podoplanin knockdown and CLEC-2 stimulation.

Our data support a model whereby podoplanin on FRCs resides in tetraspanin-enriched microdomains, and that CLEC-2 binding may orchestrate a switch between active and inactive complexes. This molecular switch allows for rapid yet reversible inhibition of contractility in FRCs, allowing the lymph node to undergo continuous cycles of remodelling during immune responses.
There is an unsolved controversy regarding the possible functional consequences of different physiological haplotypes of mtDNA in inflammatory processes. To address this issue, all mitochondrial DNAs (mtDNA) of a given cell in our organism are essentially identical, a situation termed homoplasmy. Animal models with identical nuclear genomes but with different physiological haplotypes of mtDNA provide a unique opportunity to study the functional consequences of mtDNA haplotypes in various disease states. In a recent study, Bleyer et al. have investigated the role of c-Rel in the regulation of Treg immune checkpoint in cancer. They found that NF-κB c-Rel is crucial for the regulatory T cell immune checkpoint in cancer. Moreover, chemical inhibition of c-Rel delayed melanoma growth and potentiated anti-PD-1 checkpoint-blockade therapy by impairing the Treg transcriptional program. Our data indicate that NF-κB regulates lymphomatous B-cell responses to expand ESAM$. To assess if expanded DCs were capable of mediating enhanced T cell responses, CD4+ T cells were co-cultured with splenic DCs derived from WT and Ptpn22$^{-/-}$ mice immunised with ovalbumin targeted to DC2 via receptor DCR2 (anti-DICR2-ova and spleen RBC adjuvant). Interestingly, the expansion of DCs, conferred by Ptpn22$^{-/-}$, was sufficient to induce enhanced T cell responses and GCs observed in Ptpn22 variant mice in vivo. Together these data provide a new insight into how perturbations to Ptpn22 contribute to generate pathogenic autoimmune responses by altering DC2 homoeostasis and function.

**BS.B.01.04 Mitochondrial DNA shapes metabolism in immune response**

A. V. Lechuga-Vico$^1$, G. Protéa, U. Gileadi$^3$, A. Latore-Pellicier$^1$, J. Pellico$^1$, I. Ruiz-Cabello$^5$, V. Verdurola$^1$, A. I. Enriquez$^4$

$^1$Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain, $^2$Centro de Investigación en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain, $^3$MRc Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, $^4$CIC biomaGUNE, San Sebastián-Donostia, Spain, $^5$Universidad Complutense, Madrid, Spain

All mitochondrial DNAs (mtDNA) of a given cell in our organism are essentially identical, a situation termed homoplasy. Animal models with identical nuclear genomes but with different mtDNA haplotypes (comparing mice) generate functionally different OXPHOS systems that shape the organismal metabolism$^6$, supporting the conclusion that different mtDNA haplotypes are phenotypically relevant.

There is an unsolved controversy regarding the possible functional consequences of different physiological haplotypes of mtDNA in inflammatory processes. To address this issue, we have characterized comiplastic cells throughout their lifetime through transcriptomic, metabolomic, biochemical and phenotypical studies. We have also applied in vivo imaging to study highly cytotoxic anti-angiogenic immune responses to expand ESAM$. To assess if expanded DCs were capable of mediating enhanced T cell responses, CD4+ T cells were co-cultured with splenic DCs derived from WT and Ptpn22$^{-/-}$ mice immunised with ovalbumin targeted to DC2 via receptor DCR2 (anti-DICR2-ova and spleen RBC adjuvant). Interestingly, the expansion of DCs, conferred by Ptpn22$^{-/-}$, was sufficient to induce enhanced T cell responses and GCs observed in Ptpn22 variant mice in vivo. Together these data provide a new insight into how perturbations to Ptpn22 contribute to generate pathogenic autoimmune responses by altering DC2 homoeostasis and function.

**BS.A.01.06 Autoimmune associated gene PTPN22 is a novel negative regulator of dendritic cell homeostasis and function**

H. A. Purvis$^1$, F. Clarke$^4$, G. H. Cornish$^1$, T. J. Perrie$^1$, C. Sanchez-Blanco$^1$, O. J. Rawlings$^1$, R. Zamora$^1$, P. Guermonprez$^1$, A. R. Copec$^1$

$^1$CMCBI Kings College London, London, United Kingdom, $^2$University of Washington School of Medicine, Seattle, United States, $^3$Institute of Immunology and Infection Research, Edinburgh, United Kingdom

Classical CD11c$^+$MHCII$^+$ dendritic cells (DC) are divided into functionally distinct phenotypes including the SIRPa$^+$ DC2 subset. Splenic DC2 activate CD4$^+$ T cells and are potent inducers of follicular helper T-cells (T FH ) stimulating germinal center (GC) formation. A single nucleotide polymorphism within the phosphatase PTPN22 confers an enhanced risk of predicting multiple autoimmune diseases including rheumatoid arthritis and type 1 diabetes. PTPN22 negatively regulates multiple immunoreceptor signaling cascades and Ptpn22$^{-/-}$ mice display an expanded T cell repertoire and increased GCs. We observed a specific expansion in the number and proportion of splenic ESAM$^+$ DC2 within Ptpn22$^{-/-}$ and Ptpn22$^{-/-}$ (orthologue of the human autoimmune associated variant) mice compared to WT. Competitive adoptive bone marrow transfers revealed that PTPN22 negatively regulates DC2 development in a DC intrinsic manner. PTPN22-mediated DC2 expansion occurred post-DC precursor development and was not conferred by enhanced responsiveness to Flt3L or GM-CSF. Our data indicate that PTPN22 regulates lymphomatous B-cell responses to expand ESAM$^+$ DC2. To assess if expanded DCs were capable of mediating enhanced T cell responses, CD4+ T cells were co-cultured with splenic DCs derived from WT and Ptpn22$^{-/-}$ mice immunised with ovalbumin targeted to DC2 via receptor DCR2 (anti-DICR2-ova and spleen RBC adjuvant). Interestingly, the expansion of DCs, conferred by Ptpn22$^{-/-}$, was sufficient to induce enhanced T cell responses and GCs observed in Ptpn22 variant mice in vivo. Together these data provide a new insight into how perturbations to Ptpn22 contribute to generate pathogenic autoimmune responses by altering DC2 homoeostasis and function.
Immune responses are highly variable between individuals and populations, with this variance driven by genetic and environmental factors. To better define this inherent variability and to dissect its causes the Milieu Interieur cohort was established consisting of 1,000 healthy donors stratified by age (20-70 years old) and sex (50:50). From these donors we have characterized baseline immune phenotypes by multi-parameter flow cytometry, and induced immune responses in standardized whole blood stimulations systems. We have previously shown how innate immune cells are preferentially shaped by genetics, while adaptive immune cells are more impacted by environmental factors. More recently we have defined key immune stimuli that capture the full breadth of induced immune responses and characterized these responses at transcriptional and proteome levels. We are currently integrating this rich data set with complementary cellular, genetic and epigenetic data sets in a systems immunology approach. Classical statistical approaches as well as machine learning techniques are being applied to define immune response networks and determine which components of these pathways are controlled at the genetic level. In parallel disease specific immune perturbations in infection and autoimmunity have been identified utilizing the same standardized approach. Direct comparison with our well defined 1,000 healthy donor cohort is allowing for a new understanding of these mechanisms in disease. In summary, these results will lay the foundations for the integration of immune response variability in smart clinical study design and eventually precision medicine strategies.

BS.C.01.06

T cell - target cell communication is determined by glycosphingolipid-mediated shielding of cell surface proteins

A. A. de Waard1, M. L. Jongma1, T. Zhang1, S. Holst1, M. Wuhrer1, C. E. van der Schoot1, R. M. Spaapen1; 1Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, Leiden University Medical Center, Leiden, Netherlands.

Receptor-ligand interactions are essential for immune cell function. Using state-of-the-art genome-wide screens we identified that physical accessibility of surface MHC class I (MHC-I) can be restricted by a subtype of glycosphingolipids (GLs)-so-called (neo-)lactoseries GLs. Moreover, tumor cells expressing these GLs have a reduced capacity to activate CD8+ T cells. Here, we hypothesized that also cell surface proteins other than MHC-I are shielded by GLs preventing the binding of their natural receptors. Using CRISPR/Cas9 we generated various cell lines with a different GLs composition as confirmed using mass spectrometry. These cells and control cells were fluorescently barcoded to analyze in a single well by flow cytometry. We then screened the various cell lines for new cell surface proteins with a restricted physical accessibility, just like MHC-I, using a custom monoclonal antibody array. Out of 26 proteins analyzed, the accessibility of 18 cell surface proteins was specifically reduced by the (neo-)lactoseries GL subtype. Although GLs are described to be enriched in specific membrane microdomains, both proteins within and outside of these microdomains were affected by GLs. Importantly, next to restricting accessibility of surface proteins for antibodies, the (neo-)lactoseries GLs largely blocked the ligation of the receptors LIR-1 and SIRP-a to their ligands, MHC-I and CD47 respectively.

To conclude, the GLs repertoire regulates shielding of several surface proteins which affects the interaction with CD8+ T cells. Tumors and viruses may thus specifically corrupt intercellular communication with the immune system through alterations in the cellular GL signature.

BS.C.01.07

TLR7 escapes X chromosome inactivation in cells

J. Guéry; 1BS.C.01.01

Sanquin Research, Amsterdam, Netherlands, Leiden University Medical Center, Leiden, Netherlands.

Like other autoimmune diseases, systemic lupus erythematosus (SLE) arises due to gene-environment interactions. TLR7 gene expression is XY-independent, X-linked genes are typically silenced due to X chromosome inactivation (XCI) and the gene expression is typically silenced in both males and females. Here, we report that TLR7 escapes XCI in B lymphocytes, pDCs and monocytes from females by virtue of the GLs.

BS.C.01.08

Human regulatory T cells suppress CD4+ T cells by rapidly altering the phosphoproteome

R. N. Jash1, F. Marabita2, N. Bina1, Z. Su1, A. Altman1, A. J. Heck2, J. Tegner1, A. Schmidt1; 1Karolinska Institute, Stockholm, Sweden, 2Utrecht University, Utrecht, Netherlands, 1Joela Inst. for Allergy and Immunology, La Jolla, United States.

Regulatory T cells (Tregs) control key events of immunity primarily by suppression of effector T cells, and Treg dysfunction is involved in the pathogenesis of autoimmunity, allergy and cancer. We previously revealed that Tregs rapidly suppress T cell receptor (TCR)-induced calcium store depletion in conventional CD4+CD25+ T cells (Tcons) independently of IP3 levels, consequently inhibiting NFAT signaling and effector cytokine expression. Here, we study Treg suppression mechanisms through unbiased phosphoproteomics of primary human Tcons upon TCR stimulation and Treg-mediated suppression, respectively. We demonstrated that Tregs suppress the signalling cascade in Tcons by inducing a state of overall decreased phosphorylation as opposed to TCR stimulation. Further we also discovered novel phosphoproteins that are phosphorylated in TCR signaling in Tcons. We then identified the proteins involved in TCR-dependent pathways by isolation of TCR ligated Tcons by magnetic depletion and co-precipitation with Tregs. Mutation of these DE666 phosphosites abrogated interaction of DE666 with the IP3 receptor and affected NFAT activation and cytokine transcription in primary Tcons. Additionally, we also discovered a phosphatase inhibitor from our phosphoproteomic screen, the loss of which rendered the Tcons to be resistant to Treg-mediated suppression.

This novel mechanism and phosphoproteomes data resource may aid in modifying sensitivity of Tcons to Treg-mediated suppression and contribute to improve treatment of autoimmunity and cancer, particularly considering the frequently observed resistance of target Tcons to Treg-mediated suppression in human autoimmune disease.
Prophylactic treatment with novel forms of allergen-laden virus-like nanoparticles (VNP) induces specific tolerance in a mouse model of allergy


Institute of Immunology; Center for Pathophysiology, Infectology and Immunology; Medical University, Vienna, Austria, 2Department of Nanobiotechnology, Institute for Biophysics, University of Natural Resources and Life Sciences, Vienna, Austria, 3Department of Dermatology, Laboratory of Cellular and Molecular Immunobiology of the Skin, Medical University, Vienna, Austria, 4Institute of Pathophysiology, Center for Pathophysiology, Infectology and Immunology; Medical University, Vienna, Austria.

In high-risk populations, allergen-specific prophylaxis could protect from sensitization and subsequent development of allergic diseases. However, such treatment might itself induce sensitization and allergies. Therefore, now non-allergenic vaccine formulations, such as virus-like nanoparticles (VNP) are needed. We here targeted the major mugwort allergen Art V 1 either to the surface or to the inner side of VNP by genetic engineering and subjected the vaccine candidates to biochemical and immunological analyses in vitro, as well as in a humanized mouse model of mugwort allergy in vivo. Degranulation of RBL cells sensitized with Art V 1-specific IgE occurred exclusively upon incubation to VNP expressing surface-exposed but not shielded allergen, whereas both VNP versions induced proliferation and cytokine production of allergen-specific T cells in vitro. Upon intranasal application in mice, VNP expressing surface-exposed allergen induced allergen-specific antibodies, including IgG, which was not observed for VNP expressing shielded allergen, making them promising candidates for prophylactic application. Preventive treatment with VNP expressing shielded allergen protected mice from subsequent sensitization with mugwort pollen extract. Protection was associated with a Th1/Treg-dominated cytokine response, increased Foxp3 Treg numbers in lungs and reduced lung resistance. In vivo, functional analyses demonstrated that surface-exposed mugwort macroparticles but also CD103+ DCs, which were known to be strong inducers of Foxp3+ Treg. Allergen-laden VNP represent a novel and versatile in vivo allergen delivery platform to selectively target T cells that can be used for immunotherapy without inducing de novo sensitization. Supported by Austrian Science Fund (FWF) DK-D1-148, SFB-F4609, F4605.

Macrophages require CD200 receptor to resolve inflammatory pain

R. Raadf, M. van der Vlist, H. Willemen, J. Prado Sanchez, L. Meyoard, N. Eijkelkamp; University Medical Center Utrecht, Utrecht, Netherlands.

Pain is a cardinal symptom of inflammation. The resolution of pain is presumed to be the result of discontinued inflammatory processes, yet in various diseases such as rheumatoid arthritis, diseases persist even when inflammatory processes have subsided. Here we investigated the contribution of macrophages in the regulation of inflammatory pain. Transient inflammatory pain was induced by injection of carrageenan in mouse hind paws. In this model, monocytes/macrophages infiltrated the dorsal root ganglia (DRG) that are distant from inflammation and contain the cell bodies of sensory neurons innervating the hind paw. Unexpectedly, monocyte/macrophage-depleted mice failed to resolve inflammatory pain whilst the duration of carrageenan-induced inflammation was unaffected. During transient pain, DRG-infiltrating macrophages expressed the M2 marker CD200, but not M1 marker NO. Intrathecal injection of CD11b+ monocytes, M0 macrophages, or in-vitro M2-polarized macrophages in monocyte/macrophage-depleted mice resolved inflammatory pain, whilst M1 macrophages were unable to resolve pain. M2 Macrophages express high levels of the immune inhibitory CD200-Receptor (CD200R). Similar to macrophage-depleted mice, CD200R-/- mice failed to resolve inflammatory pain. Resolution of inflammatory pain required CD200 expression on monocytes/macrophages. Therefore, administration of WT, but not CD200-/- mice restored the capacity to resolve inflammatory pain.

In conclusion, we show that monocytes/macrophages require CD200R to drive the resolution of inflammatory pain in the DRG, and thereby prevent development of chronic pain. These data indicate that monocyte/macrophage function extends beyond control of inflammation to the regulation of pain-sensing neurons.

Tissue-resident memory CD8+ T cells form systemic effector and memory responses but ‘remember’ their site of origin

F. M. Behr, T. H. Wesselinck, L. Pargo Vidal, N. A. Krauten, R. Stark, K. P. van Gisbergen; St. Gallen Research and Landsteiner Laboratory, Amsterdam, Netherlands.

Tissue-resident memory CD8+ T cells (TRM) are non-circulating memory T cells localized to peripheral (barrier) tissues. TRM provide efficient early protection against local reinfection through rapid cytokine production and local proliferation. However, the contribution of TRM to systemic secondary effector and memory responses remains unclear.

In order to investigate T RM responses after re-challenge in vivo, we established an adoptive transfer model of memory CD8+ T cells arising after acute viral infection. Intestinal T RM and circulating effector memory (T EMR) and central memory CD8+ T cells (T CMR) were isolated from immune mice, and co-transferred into naive recipients. Unlike their circulating counterparts, T CMR cells were confined to non-lymphoid peripheral tissues following transfer and retained a resident phenotype. After viral challenge, T CMR cells, similar to circulating memory subsets, gave rise to a systemic recall response, albeit of reduced magnitude. Upon viral clearance, T CMR formed circulating and resident secondary memory cells, but did not give rise to T RM cells. Interestingly, the intestine-derived T CMR were superior at re-generating secondary T RM in the intestinal compartment, but not at other sites. This was consistent with their selective re-expression of the intestine-homing receptor CCR9. In contrast, re-activated liver T CMR lacked the capacity to access the intestine, preferentially accumulated in the liver and upregulated receptors associated with liver-homing.

Our findings demonstrate that T RM have the potential to generate body-wide effector and memory responses, but retain an intrinsic preference for their tissue of origin. This may pose important implications on future cell therapy and vaccination strategies that employ T RM.

Bright Sparks D

Intestinal IgA shows specific broad binding to phylogenetically non-related bacteria

J. Kabbert, H. Wardemann, M. Hepworth.

Secretory immunoglobulin A (SIgA) is a key component in gut homeostasis. SIgA binds to luminal and gut epithelial surface associated bacteria, thereby contributing to intestinal infection, it remains elusive how the host immune system can generate and regulate beneficial SIgA responses to a highly dynamic commensal setting.

These data indicate that monocyte/macrophage function extends beyond control of inflammation to the regulation of pain-sensing neurons.

Secretory IgA may contribute to host defense by pathogens and maintain a stable microbiota composition. Considering the vast changes in the microbiota consortium depending on diet, medical treatment and infection, it remains elusive how the host immune system can generate and regulate beneficial SIgA responses to a highly dynamic commensal setting.

This study sought to profile the binding spectrum of monoclonal IgA antibodies (mABs) derived from human healthy donors and inflammatory bowel disease (IBD) patients by flow cytometry. Screening of almost 200 monoclonal IgA antibodies revealed an unexpected high frequency of mABs with substantial microbiota reactivity. In order to determine the binding spectrum of microbial reactive mABs, we used these mABs to stain bacteria isolated from RAG-/- feces with subsequent 16S rDNA sequencing of bound and unbound bacterial fractions. Individual mAbs bound a diverse spectrum of commensals rather than showing reactivity to single taxa. This suggests that single monoclonal mAbs functionally bind a relevant fraction of different intestinal bacteria in vivo. Unlike recent reports, we did not observe a correlation of high microbiota reactivity and polyreactivity of respective mAbs. In addition, while mAbs with microbiota reactivity showed frequent somatic mutations, germ-line variants of previously high binding mAbs showed decreased microbiota binding or an altered binding profile. We therefore speculate that ongoing somatic hypermutation selects for intestinal IgA with broad, yet specific binding to different bacterial taxa.
BRIGHT SPARKS WORKSHOPS

BS.D.01.03

Eomes broadens the scope of DCD T cell memory by inhibiting apoptosis in low-affinity cells


1School of Medicine, University of Rijeka, Rijeka, Croatia; 2Dept. of Experimental Immunology, AMC Amsterdam, Amsterdam, Netherlands; 3Dept. of Clinical Immunology & Rheumatology, AMC Amsterdam, Amsterdam, Netherlands; 4Center for Hematology and Regenerative Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden; 5Institute for Virology and Research Center for Immunotherapy (FZI) at the University Medical Center of the Johannes Gutenberg University, Mainz, Germany; 6Dept. of Immunopathology, Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands; 7Dept. of Oncogenomics, AMC Amsterdam, Amsterdam, Netherlands.

The memory DCD T cell pool must select for high affinity clones to efficiently counter re-infection, yet must retain a level of clonal diversity to allow recognition of pathogens with mutated immune-dominant epitopes. How this is mediated is unclear, especially in the context of a selective drive for antigen-affinity. We find that two distinct mechanisms of memory cell selection operate. Using an inducible system in which we can time elimination of Eomes by Polycl(TC) injection we show that low-affinity memory exclusively depends on the transcription factor Eomes in the first days after antigen encounter. Eomes is induced at low activating signal strength and directly drives transcription of the pro-survival protein Bcl-2. At higher signal intensity T-bet is induced which suppresses Bcl-2, generating a survival advantage for low-affinity cells. In contrast, high-affinity cells form memory independent of Eomes, but have a proliferative advantage over low-affinity cells, which compensates for their survival deficit. The Eomes-deficient DCD T cell memory population therefore lacks low-affinity cells, resulting in a strongly reduced capacity to target antigen with point mutations in its immune-dominant epitope. By specifically targeting Bcl-2 with a small molecule inhibitor we were able to selectively drive memory cell selection and in this sense we could increase the specificity of the DCD T cell response. In summary, we demonstrate on a molecular level how sufficient diversity of the memory pool is established in an environment of affinity-based selection. Moreover, we demonstrate that the Eomes/Bcl2 axis may be exploited therapeutically to modify the scope of T cell based vaccines.

BS.D.01.04

Pentraxin3 Regulates IL-17A Mediated Immunity to Leishmania

G. Gupta, P. Jia, J. E. Uzonna;
University of Manitoba, Winnipeg, Canada.

Cutaneous leishmaniasis (CL), caused by the protozoan parasite Leishmania (L) major, results in ulcerative skin lesions at the sites of infection. Studies show that the nature of immune response plays a crucial role in resolution of skin lesions during infection. The long Pentraxin 3 (PTX3), a soluble pattern recognition molecule, is critical for wound healing by regulating tissue repair and innate and adaptive responses during infection and inflammation. Here, we show that PTX3 contributes to susceptibility to CL. PTX3 +/- mice were highly resistant to primary and secondary L. major infections. Interestingly, the enhanced resistance of PTX3 +/- mice to L. major was not associated with enhanced IFN-γ or decreased IL-4 response. Instead, L. major-infected PTX3 +/- mice displayed strong IL-17 response and in vivo neutralisation of IL-17A abolished their enhanced resistance and resulted in elevated parasite burden compared to their untreated controls. In in vitro polarization studies, more naïve CD4+ T cells from PTX3 +/- mice significantly differentiated into Th17 cells compared to those from WT mice. This was associated with increased expression of Th17-specific transcription factors like RORγt and STAT3. Addition of recombinant PTX3 into Th17 polarizing cultures of PTX3 +/- CD4+ T cells led to significant reduction in the expression of Th17-specific transcription and the frequency of Th17 cells. Collectively, our results show that PTX3 contributes to pathogenesis of CL by negatively regulating inflammation via enhancing IL-17 response.

BS.D.01.05

Imaging the host-pathogen interaction in tuberculosis in a bioelectrospray 3D cell culture model


1University of Southampton, Faculty of Medicine, Southampton, United Kingdom; 2University of Southampton, Chemistry, Southampton, United Kingdom.

Introduction: Tuberculosis is a deadly infectious disease caused by the bacterium Mycobacterium tuberculosis (Mtb). Traditional animal models as well as conventional ‘two-dimensional’ cell cultures do not accurately mimic human tuberculosis infection in vivo, as the formation of caseating granulomas and degradation of extracellular matrix. Materials and Methods: We study a bioelectrospray-generated 3D cell culture model of tuberculosis, using diverse imaging techniques. In the 3D culture, Mtb-infected BMDCs (murine; H37Rv) are mixed with an alginate-collagen gel. We investigated the host-bacteria interaction at high resolution at various stages of infection using Transmission Electron Microscopy (TEM). We used Micro-Computed Tomography (µCT) to show distribution of BMDCs in 3D in comparison to a human tuberculous lung biopsy. This was then correlated to traditional H&E, and matrix staining of the sectioned lung block. Using label-free microscopy (Coherent Anti-Stokes Raman Scattering (CARS), Second Harmonic Generation (SHG)), BMDC aggregation and collagen fibers were imaged.

Results: More lipid bodies were detected by TEM in the Mtb-infected samples than the uninfected controls. TEM and SHG imaging revealed collagen fibres attached to the surface of BMDCs. CARS microscopy showed that infection with Mtb, as well as the presence of collagen in the 3D matrix, influence the number of BMDC aggregates forming with the 3D culture. Preliminary data from µCT indicate that this technique can provide quantitative data on BMDC aggregates in 3D that can be cross-correlated with human biopsies.

Conclusion: A combination of traditional and emerging imaging modalities can provide new insight into the host-pathogen interaction in tuberculosis.

BS.D.01.06

Imaging cell heterogeneity reveals functional heterogeneity within plasmacytoid dendritic cells and identifies environmental cues that drive type I IFN production


1Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, Netherlands; 2Lab of Immunooengineering - Dept. Biomedical Engineering - Eindhoven University of Technology, Eindhoven, Netherlands; 3Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, Netherlands; 4Department of Gastroenterology & Hepatology - Institute of Liver Sciences, Royal Netherlands Academy of Arts and Sciences (KNAW) and University Medical Center Utrecht, Utrecht, Netherlands; 5Laboratory of Immunology - Department of Laboratory Medicine - Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, Netherlands; 6Laboratory of Hematology - Department of Laboratory Medicine - Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, Netherlands; 7Laboratory of Physical Organic Chemistry, Institute for Molecules and Materials, Radboud University, Nijmegen, Netherlands.

Introduction: Cellular heterogeneity emerges as a key feature of type I IFN-mediated antiviral immunity. Little is known about the factors involved in modulating cellular heterogeneity and the influence of the microenvironment. We investigate how cellular heterogeneity and the microenvironment orchestrate the type I IFN response in plasmacytoid dendritic cells (pDCs). Materials and Methods: We developed a droplet-based microfluidic platform and investigated type I IFN production in human pDCs at single-cell level. Furthermore, our platform warrants functional analysis of live single cells under omission of a microenvironment and we combined this with single cell RNA-seq. Results: For the first time in an unbiased approach, we show here in primary human immune cells that an additional deeper level of functional heterogeneity arises upon stimulation of dendritic cells. We demonstrate that type I IFN production by primary human pDCs is stochastically regulated when stimulated individually with pathogen analogues. Less than 1% of pDCs produced type I IFN despite secretion and expression of other activation markers. Importantly, we determined a crucial role for the microenvironment as a paracrine feedforward loop to amplify the type I IFN production leading to the well-described robust type I IFN response that pDCs are so famous for. Conclusions: Our unique technology platform revealed that the inflammatory state of the microenvironment and not the stimulus concentration perceived by pDCs determines the response strength. This has important implications for pDC-focused treatment approaches as the main focus of regulation should be in modifying the type I IFN-based paracrine regulatory system.
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WS.A1.01.01 Myeloid lineage specifications

R. Gentek
Centre d’Immunologie de Marseille-Luminy (CIML), Marseille, France.

Hematopoiesis occurs in spatio-temporally distinct waves. The aorta-gonado-mesonephros (AGM) gives rise to ‘definitive’ hematopoietic stem cells (HSC) with the potential for all blood lineages. At earlier stages, hematopoietic progenitors emerging in the yolk sac (YS) generate erythrocytes and megakaryocytes, while YS derived erythro-myeloid progenitors give rise to macrophages (Mac). Whether HSC independent YS progenitors also significantly contribute to other lineages is currently unknown. This is at least partially due to the limitations of current lineage tracing models. Here, we established a novel model that enables fate mapping of both HSC independent YS and definitive hematopoiesis in an efficient and precise, temporally defined fashion (Cdh5-CreERT2 fate mapping). Using this tool, we revealed that embryonic mast cells (MC) initially derive from YS precursors, but get progressively replaced by definitive MC. Replacement of YS derived MC occurs with tissue specific kinetics: YS derived embryonic and definitive adult MC differ substantially, phenotypically and transcriptionally. Moreover, adult MC are largely independent from the BM at steady state and during replenishment following depletion. These findings challenge the current dogma that MC originate from the BM. Instead, their developmental kinetics are highly reminiscent of Mac. Our work adds MC to the list of lineages with dual origin. Key questions that have remained unanswered or controversial for other lineages thus also apply to MC, such as the relative influence of ontology and (micro)environment and the establishment of immune cell niches. Cdh5-CreERT2 fate mapping represents a powerful tool to address these questions and further dissect hematopoiesis.

WS.A1.01.02 Determining dendritic cell ontogeny across organs by cellular barcoding

T. Tak, A. Magniez, L. Perié
Institut Curie, Paris, France.

Dendritic cells (DCs) are rare cells that are widely distributed throughout the body. A multitude of functions, origins and cellular markers have been attributed to DCs, resulting in a complicated classification. We aimed to determine the ontogeny of the different DC subsets across different organs in the mouse using cellular barcoding, a technique that simultaneously traces the differentiation of many cells in vivo. Murine hematopoietic stem and progenitor cells (HSCs and MPPs) were infected with a lentivirus containing DNA barcodes of 100 random nucleotides. They resulted in uniquely barcoded progenitors whose progeny inherit the barcode. HSCs/MPPs were transferred into sub-lethally irradiated C57BL/6 mice. After 2-6 weeks, DC subsets (pDC, cDC1 and cDC2), B cells and neutrophils were isolated from bone marrow, spleen liver and lungs. Barcodes were identified by PCR and deep sequencing. Since daughter cells from the same progenitor share the same barcode, ontogeny could be analysed by hierarchical clustering. After HSC transplantation, detected barcodes were shared across a wide range of cell types, indicating that a single HSC can produce a wide variety of cells. Samples obtained after transplantation of MPPs showed a large proportion of barcodes being shared between different DC subsets within each organ and between DC subsets in liver and lungs. Only few barcodes were shared between DC subsets from spleen and liver/lungs, however, suggesting a different developmental origin of DC subsets in lungs and liver compared to the spleen.

WS.A1.01.03 Bone marrow-resident dendritic cells play an important role in anti-fungal immunity by boosting granulopoiesis upon recognition of yeast particles

M. Goedhart, E. Slot, M. F. Pascutti, S. Geerman, T. Rademakers, B. Noto, C. Voermans, M. A. Nolte
Sanquin, Amsterdam, Netherlands.

Systemic infections with yeast and fungi are major causes of morbidity and mortality following hematopoietic stem cell transplantations and in patients with bone marrow (BM) failure. As BM is enriched for memory T cells specific for fungal antigens, we hypothesize that BM is important for anti-fungal immunity. Here, we focused on dendritic cells (DCs) in murine BM, which largely belong to the IRF4-regulated subtype that is associated with Th1/Th17 immunity. We found that these cells are localized around sinusoids and rapidly activated upon intravenous endotoxin injection. Gene-expression profiling revealed that BM-resident DCs are, compared to the splenic counterpart enriched for several c-type lectins, including Dectin-1, which can bind beta-glucans expressed on fungi and yeast. Indeed, DCs in BM were much more efficient in phagocytosis of both yeast-derived zymosan-particles and conidia of Aspergillus compared to their splenic counterparts, which was highly dependent on Dectin-1. DCs in human BM were also able to efficiently take up zymosan, which depended on β1-integrins. Given their localization inside the body’s hematopoietic organ, we examined whether BM-resident DCs can also regulate hematopoiesis. Strikingly, we found that zymosan-stimulated BM-resident DCs enhanced the differentiation of hematopoietic progenitor cells towards neutrophils, while also boosting the maintenance of functional progenitors. Our findings demonstrate that BM-resident DCs play an important role in anti-fungal immunity. The ability of BM-resident DCs to boost granulopoiesis is highly relevant from a clinical perspective, and contributes to our understanding of the susceptibility for fungal infections under conditions of BM damage.

WS.A1.01.04 Common monocyte progenitors are novel antimycobacterial effector cells

P. Hennke, A. Lösslein
CCI, Freiburg, Germany.

Mycobacterial tissue infections are characterized by the formation of a multicellular granuloma containing specialized immune cells. Granulomas compromise a unique macrophage species, so called multineutriculated giant cells (MGC). In this work we dissected the origin of MGC, which has remained largely exclusive, so far. Accordingly, we isolated different monocyte precursor subsets from murine bone marrow (BM) and found the common monocyte progenitor (cMoP) to have the highest potential to form MGC in response to mycobacterial glycolipids or whole bacteria. Next to the established high proliferative activity, cMoP showed striking effector cell characteristics, e.g. robust formation of TNFα and nitric oxide in response to mycobacterial glycolipids. Furthermore, cMoP showed a distinct differentiation pattern in vitro. They rapidly downregulated CD117 expression and upregulated CD11b and F4/80 expression, which is in line with the differentiation into macrophages. However, in contrast to the immunophenotype of differentiated macrophages they maintained high potential to form MGC. Transcriptome analysis revealed an increase in cholesterol and fatty acid metabolism in cMoP, representing a metabolic profile, which allows for the formation of lipid bodies and nucleation of mycobacteria in the cytosol. Fatty acid synthase inhibition impaired MGC formation by cMoP, indicating that the metabolic changes are a prerequisite for the transformation program. We hypothesize that cMoP serve as MGC progenitors in local mycobacterial infections. Together, we herewith provide firm evidence that cMoP, which have been hitherto defined as precursors committed to renew monocytes only, act as specific effector cells in antimycobacterial immunity.

WS.A1.01.05 Identification of the unique functional phenotype of IMATE-defining monocytes that drive hepatic T cell proliferation

K. Pawelka1, P. Knolle1, M. Heikenwälder1
1Institute of Molecular Immunology, Munich, Germany, 2DKFZ, Heidelberg, Germany.

Intrahepatic myeloid-cell aggregates form in response to Toll-like-receptor 9 (TLR9) signaling in a TNF-dependent fashion to provide a unique anatomic structure that drives local proliferation of cytotoxic CD8 T cells (IMATEs) and confers protection against viral infection. Yet, the identity of the IMATE-defining myeloid cell population remained elusive. We systematically analyzed the phenotype of myeloid cells in the murine liver upon TLR9 activation using a set of different methodologies. Initial flow cytometric phenotypic characterization and tSNE analysis revealed a complex composition of monocytes and newly differentiating macrophages that hinted towards a sequential replacement of liver-resident macrophages following by repopulation through bone marrow derived inflammatory monocytes. Laser-capture microdissection and genome-wide analysis of gene expression identified a set of marker proteins that were validated by flow cytometry and led to the definition of a particular phenotype of monocyte-derived macrophages exclusively found in IMATEs but not elsewhere.

Functional assays of these IMATE-defining monocyte-derived macrophages revealed a high potency in the induction of CD8 T cell proliferation, in the differentiation towards GzmB expression rendering them efficient killer cells and in cross-presenting soluble antigens to CD8 T cells. Liver macrophages, in contrast, failed to provide any support for T cell proliferation and did not show significant cross-presentation capacity. The transient presence of IMATE-defining monocyte-derived macrophages in the liver indicates that protective hepatic T cell immunity is determined by the dynamics of the changes in inhibitory vs stimulatory macrophage populations, which do not fall into the conventional M1/M2 categories but are related to IMATE formation.
Institute of Immunology and Immunotherapy, Birmingham, United Kingdom, Fred Hutchinson Cancer Research Center, Seattle, United States.

Introduction Cytomegalovirus (CMV) elicits a strong T cell immune response which increases during aging in a process termed ‘memory-inflation’. CMV downregulates HLA-A/B molecules on the surface of CMV-infected cells to limit presentation of viral peptides to T-cells. Comparatively, HLA-C is expressed to a lesser extent and engages with inhibitory KIR receptors to reduce lysis by NK cells. Methods The magnitude and functional properties of CMV-specific HLA-C-restricted T-cell specific for HLA-C-restricted peptides were investigated in a cohort of 53 donors aged 23–91 years. This was achieved via peptide stimulation of PBMCs followed by multi-colour flow cytometry.

WS.A2.01.02 Peripheral Antigen T cells are affected by highly differentiated, senescent, and exhausted T cells, and pro-inflammatory changes in the human Bone Marrow

I. M. Ogunsulire1, M. Almanara1, D. Hildemanb1, C. Hölscherb1
1Division of Immunology, Research Centre Borstel, Germany, Südfeld, Germany, 2Division of Immunobiology, Cincinnati Children’s Hospital Medical Centre, USA, Cincinnati, United States.

One of Mankind’s greatest triumphs is prolonged life-expectancy. Conversely, this increase in lifespan is accompanied with its own set of problems. One such problem is a weakened immune system, which leads to the increased susceptibility to infections and diseases in the elderly. A major component of immune dysregulation is chronic inflammation, referred to as ‘inflammaging’, and is characterised by elevated systemic level of interleukin (IL)-6. However, mechanisms controlling inflammaging and its effects on the immune system remain unclear. Recent data show that IL6 promotes the accumulation of FoxP3+ CD4+ T cells which produce a major anti-inflammatory cytokine, IL-10. So far, the origin and the type of IL-10-producing cells are yet to be determined. Based on cell described in the literature, we envisioned four potential origins of these IL-10-producing cells, namely exTh17, exTregs, type I regulatory (Tr1) cells and T follicular helper (Tfh) cells. Using IL-17 fate mapping reporter mice, we found that roughly 2% of the IL-10-producing cells were exTh17 cells. Again, using FoxP3 fate mapping reporter mice, roughly 25% of the IL-10-producing cells were exTfh cells, although this frequency was similar in young and old mice.

Strong homeostatic TCR signals induce formation of self-tolerant virtual memory CD8 T cells

A. Moudra1, J. Zuo2, H. Pearce3, S. Ridge5, P. Moss5
1Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic, 2Department of Biomedicine, University Hospital and University of Basel, Basel, Switzerland, 3Swiss Institute of Bioinformatics, Basel, Switzerland, 4Swiss Vaccine Research Institute, Epalinges, Switzerland, 5Division of Animal Physiology and Immunology, School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany, 6Department of Clinical Research (DKF), University of Bern, Bern, Switzerland, 7Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, Canada.

Many antigen-experienced immune cells migrate back to the bone marrow (BM), where they can remain in BM niches for an extended period. In this study, a detailed phenotypical and functional characterization of immune cells isolated from human BM was analyzed to determine if the accumulation of highly differentiated cells limit the accumulation of other immune cells. CD8 T cells which no longer express the CD28 co-stimulatory molecule have acquired the expression of replicative senescence markers CD57 and/or KLRG-1, increase in the BM with age. Exhausted PD-1+ T cells are also seen to increase in the BM with age. Senescent cells secrete pro-inflammatory cytokines inducing low-grade chronic inflammation. Using BM and peripheral blood samples from patients undergoing hip replacement surgery, we show that highly differentiated CD8+ T cells in the BM negatively correlated with B cells, and similar correlations were seen for highly-differentiated, pro-inflammatory cytokine-producing CD8+ T cells. In addition, mRNA expression of IL-15 and IFNγ negatively correlated with B cells in the BM. Peripheral Dipherthia antibody titers negatively correlated with highly-differentated CD8+ T cells, and Exhausted Central Memory CD8+ and CD4+ T cells in the BM. Senescent CD4+ and CD8+ T cells in the BM and PB, as well as ROS production in the BM, also negatively correlated with the Dipherthia Ab titer. In summary, the accumulation and maintenance of highly-differentiated, senescent, and exhausted cells in the BM may negatively effect the maintenance of other immune cell types, and therefore reduce overall immune function.
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**WORKSHOPS**

**WS.A2.01.05**

Age-Related Decline in Primary CD8+ T Cell Responses is Associated with the Development of Senescence in a Subset of Antigenically Naive CD8+ T Cells


1Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Clayton, Australia; 2The Doherty Institute for Infection and Immunity and Department of Microbiology and Immunology, University of Melbourne, Melbourne, Australia; 3Monash Biomedicine Discovery Institute and Department of Medicine, Monash University, Clayton, Australia; 4Bioinformatics Platform, Monash University, Clayton, Australia.

Aging undermines primary CD8+ T cell responses and this occurs, in part, due to direct effects on naïve CD8+ T cells to reduce intrinsic functionality, but the precise nature of any intrinsic defect and its molecular basis remains to be defined. Ageing also causes accumulation of antigen-naïve but semi-differentiated “virtual memory” (Tvm) cells, recently identified in humans, but their contribution to age-related functional decline is unclear. Here, we show that Tvm cells become nearly completely non-proliferative in aged mice and humans, despite being highly proliferative in young individuals. In contrast, conventional naïve T cells (Tcn) retain almost complete proliferative capacity in both aged mice and humans. Adaptive transfer experiments in mice illustrated that the proliferative dysfunction acquired by Tvm cells was imposed by the aged environment and could not be rescued by maintenance in a young environment. Despite previous studies to the contrary, transcriptional analyses did not imply exhaustion in aged Tvm cell dysfunction. Rather, these cells exhibited a profile consistent with senescence, with increased Bcl-2 expression and phosphorylation of γ-H2AX in steady state, along with increased Cdkn1a (p21) expression and defective cyclin D1 accumulation after TCR stimulation. Collectively, this study marks the first description of senescence in an antigenically naïve T cell population, and highlights markedly different impacts of ageing on distinct T cell populations. Consequently, this work has implications for the targeting of distinct T cell populations in current immunotherapies.

**WS.A2.01.06**

Influence of aging on calcium signals and cytotoxicity in murine CD8+ T cells

A. Angenendt, R. Steiner, A. Knöckel, G. Schwaig, E. Krause, A. Lis

1Biophysics, CIPMM, Saarland University, Homburg, Germany; 2Physiology, CIPMM, Saarland University, Homburg, Germany.

Cytotoxic T lymphocytes (CTLs) are key players in the adaptive immune response and several steps of the CTL killing machinery require or are modulated by Ca2+ itself. The major route of Ca2+ influx in lymphocytes is through store-operated calcium entry (SOCE). CTL function is altered in vivo and in vitro in elderly compared to adult individuals and the immune system is compromised with progressing age. It is here investigated, whether reduced expressions of SOCE components, stromal interaction molecule (STIM) and Orai, contribute to Ca2+ signal reductions in CD8+ T cells from elderly mice, we performed flow cytometry, electrophysiology and molecular biology experiments with murine CD8+ T cells from an adult and an elderly age group. Furthermore, we compared their killing kinetic and efficiency using a time-resolved killing assay, investigated the Ca2+-dependency of the process and questioned the depletion of relevant proteins involved in cytotoxicity. We were able to link expression of Ca2+ signals and Ca2+ release-activated Ca2+ (Crac) currents in CD8+ T cells from elderly mice to a decrease in mRNA and protein levels of STIMs and Orais. Moreover, the reduced Ca2+ signals of stimulated CD8+ T cells from elderly mice are not due to differences in subtype distribution between both age groups, but rather in the most abundant CD8+ T cell subtypes, central and effector memory. Strikingly, we found that CD8+ T cells from elderly mice show an altered expression pattern of relevant proteins involved in killing machinery correlated with improved cytotoxicity but minor Ca2+-dependency.

**WS.A2.02.01**

Dynamic transcriptome-proteome correlation networks contribute to human myeloid differentiation and neutrophil development


1Department of Plasma Proteins, Sanquin Research, Amsterdam, Netherlands; 2Department of Blood Cell Research, Sanquin Research, Amsterdam, Netherlands; 3Department of Haematology, University of Cambridge, Cambridge, United Kingdom.

Neutrophils are the most abundant leukocytes in human blood and form the frontline of the innate host-response to bacterial and fungal infections. To fulfill this role, they exert a wide array of pathogen eliminating actions. During neutrophil development, transcriptional programs and protein production are induced to enable these actions. Using mass spectrometry-based quantitative proteomics combined with previously transcriptomics data, we unraveled the dynamic transcriptome and proteome changes that accompany the metamorphosis from four developmental stages in the bone marrow into a mature distinct non-dividing polymorphonuclear blood cell. We identified 2429 transcript-protein pairs that were differentially expressed on either RNA, protein or on both levels, which were used to assemble a co-expression network comprising of 12 modules. These included transcript-protein dynamics following the classical dogma of protein levels lagging changes in RNA expression. In contrast, we also observed modules with discordant dynamics, e.g. increased RNA expression at the final stages of differentiation, with no changes in protein level. Importantly, the transcript-protein dynamics of most modules could directly be linked to functional aspects of neutrophil development and mature neutrophil functions; such as: modules containing decreased mitochondrial process, increased immune response signatures; or modules exhibiting high association with specific neutrophil granules. Therefore, this comparison of proteome with transcriptomic data unveiled highly dynamic and differential interactions between RNA and protein kinetics during human neutrophil development linked to functional aspects of myeloid development and the typical end-stage blood neutrophil features including morphology and killing activities.

**WS.A2.02.02**

CircRNA expression is a highly regulated process during hematopoiesis and lymphocyte differentiation

B. P. Nicolet, S. Engels, F. Agilaloro, E. van den Akker, M. van der Lindern, M. C. Wolkers

Sanquin Research, Amsterdam, Netherlands.

Lymphocyte differentiation during hematopoiesis is dependent on micro-RNAs and long non-coding RNAs that - in addition to transcription factors - drive the cellular differentiation. Circular RNA (circRNA), another recently identified type of RNA, was also shown to determine vital cellular functions in many cell types. Whether and how circRNAs regulate immune cells is not yet described. Here we investigated the circRNA expression in the hematopoietic tree. Clustering based on circRNA expression recapitulated the differentiation stages in hematopoiesis. Furthermore, correlating the circular with the linear RNA expression revealed that circRNA usage is differentially regulated during hematopoiesis. Interestingly, circRNA expression is significantly higher in lymphocytes than in other hematopoietic cells. Analysis of circRNA expression in naïve and memory CD4+ and CD8+ T cells revealed higher circRNA expression in naïve than in memory T cells. Further, we found that CD4+ and CD8+ maturation is marked by 100’s of differentially expressed circRNA. We show here for the first time that circRNAs are differentially expressed during hematopoiesis and T cell differentiation, which points to a novel regulatory layer in lymphocyte differentiation.

**WS.A2.02.03**

IL-2 vs. IL-15-dependent intrathymic development of regulatory T lymphocytes

C. Apert, N. Mizonetto, P. Ramagnoli, J. van Meerwijk

1INSERM UMR1043 Centre de Physiopathologie Toulouse Purpan, TOULOUSE, France; 2Keel University, Staffordshire, United Kingdom.

Natural regulatory T cells (nTreg) develop in the thymus and are defined by the expression of the master regulator transcription factor Foxp3 as well as by high self-reactivity, which enables them to prevent autoimmune diseases. Peripheral nTreg are heterogeneous. The distinct Treg-subsets display different degrees of self-reactivity, express different transcription factors and chemokine-receptors, and inhibit peripheral immune-responses using distinct effector mechanisms. Among the various signals controlling Treg development in the thymus, the γ- cytokines IL-2 and IL-15 apparently play an important role in the survival of these cells. We investigate the roles of these two cytokines in the differentiation of distinct nTreg subsets. By using IL-2- and IL-15-deficient mice harboring a Rag2 GFP transgene that allows discrimination between developing Tregs from peripheral mature cells that had recirculated to the thymus, we confirmed that these two cytokines play quantitatively important and non-redundant roles in the development of Tregs. Treg development is decreased in absence of one or the other cytokine and virtually abolished in absence of both. Single cell RNAseq and multicolor cytometry analysis revealed that these cytokines also have a qualitative effect on Tregs favoring development of in part distinct Treg subsets. Currently we are assessing the Treg repertoire and immunosuppressive function of Tregs that develop in absence of either of the two cytokines. A better understanding of Treg-development will be of a great importance for targeting these cells in various diseases where they play a beneficial or deleterious role.
Recent thymic emigrants in neonates and adults share an antimicrobial molecular signature


1Welcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom, 2Cambridge Neuroscience, University of Cambridge, Cambridge, United Kingdom.

The neonatal immune system has to balance tolerance to commensal antigens and yet be able at this critical time to fight pathogenic infections. Importantly, owing to the large and active thymus at birth as opposed to adults, neonatal naive T compartment consist mostly of recent thymic emigrants (RTEs). However the role that recent thymic emigrants have in the early life period is still relatively unexplored. We studied 20 adult donors to achieve 80% power to detect 2-fold differences in gene expression between highly-purified naive T cell subsets of different cellular age within each donor i.e. the youngest T cells were RTEs and the oldest were naive T cells that have spent decades outside the thymus. A unique gene-expression signature in RTEs was confirmed by FACs protein investigation of 389 donors ex vivo and in functional studies including RNA-seq and FACs analysis of activated RTEs from the blood of babies, children and adults. We also studied RTEs arising directly from the thymus post lympho-depletion. We showed that complement receptors CR1 and CR2, which are known to bind complement C3b- and C3d-decorated microbial products, are hallmarks of RTEs. Most naive T cells also express transcripts encoding the UPS degrading enzyme, ADAM, and TL1A. Following activation RTEs in babies and adults produce IL-4 (CXCL8), a major chemotaxant for neutrophils in bacterial defense. In conclusion, although RTEs retain the adaptive arm of the immune system, these cells have innate, anti-bacterial functions that could help orchestrate the balance between neonatal tolerance and microbial defence.

Characterising the maturation of T cell polarisation in preterm and term infants and in the neonatal chronic lung disease bronchopulmonary dysplasia


1Monash University, Clayton, Australia, 2Monash Newborn, Australia, 3Mercy Hospital, Heidelberg, Australia, 4Royal Women's Hospital, Parkville, Australia, 5Hudson Institute, Melbourne, Australia.

Background: Bronchopulmonary dysplasia (BPD) is a common neonatal lung disease that is underpinned by pulmonary inflammation. Despite extensive research, a dominant immune pathway that drives BPD remains elusive.

Methods: Cord and peripheral blood was collected from: i) infants born at 24-29 gestational weeks at birth, on days 1, 7 and 14, and at 36 weeks gestational age; ii) healthy term infants at birth and at 4-16 weeks of age; iii) healthy adults. Following overnight stimulation with PMA-ionomycin or vehicle, the Th2/17 and regulatory T cell (Treg) subtypes were enumerated by flow cytometry. Results were analysed against BPD status and clinical events such as respiratory support, sepsis and medication.

Results: 51 preterm infants, 20 term infants and 5 adults were enrolled, and 258 unique samples collected. Th2 polarization predominated in preterm and term infants up to 16 weeks of age, with up to 62% of CD4+ T cells Th2-polarised vs 2% in adults. Baseline Th1 and Th17-polarisation was low in all groups; inducibility of Th1 and Th17-polarisation developed at 16 weeks of age. The percentage of Th1 was 5-fold higher in infants than in adults. The 36 preterm infants who suffered from BPD exhibited up to 36-fold increased frequencies of Th2-polarised T cells at most timepoints.

Conclusions: Our study sheds light on the maturation of the immune system in preterm and term infants. The severe chronic lung disease BPD is associated with strong Th2 polarization, suggesting that therapies targeting the Th2 pathway may offer benefit to the tiny BPD patients.

Immune cell aging and differentiation

WS.A2.03.01

Identification of age-related immune signatures by mass cytometry

J. Braun, S. Schlischeiser, A. Andrejewski, F. Popatzik, B. Kruse, S. Greisler, A. Arampatzis, A. Theil; 1

1Berlin-Brandenburg Center for Regenerative Therapies, Berlin, Germany.

Human biological aging is associated with functional physiological deteriorations and increasing systemic inflammation and many cellular, immunological alterations have been reported. Due to technical limitations, these reports focused mostly on specific cell populations, e.g. only T cells were assessed. However, for a systematic understanding of age-related immune signatures, all different cell subsets should be evaluated and associated with medical and functional physiological parameters. Therefore, we have performed an integrated study combining mass cytometry, multiplex ELISA and biomechanic measurements to characterize cohorts of healthy young, healthy elderly and frail elderly participants. Fresh PBMCs were analyzed by mass cytometry (37 antibodies). We applied an analysis pipeline for Automated Comprehensive Immunophenotyping and Subset Enumeration (ACISE). Here, cell populations are identified by over-clustering using SPADE, followed by characterization based on user-defined cutoffs for marker positivity, and annotation according to user-specified target phenotype definitions as well as subset hierarchies (similar to conventional gating analysis). We identified not only already reported fluctuations in CD8 TEBM, CD4 recent thymic emigrants, or non-classical monocytes, but also changes in regulatory T cells, NK cells and B cells. Some changes were exclusively and significantly observed within frail participants. Our study may represent the first comprehensive mass cytometric analysis of immune signatures including clearly separated cohorts of frail and healthy elderly. Furthermore, the applied ACISE analysis pipeline is a helpful tool for the analysis of multi-dimensional single cell data, as it allows semi-automated categorization of cell subsets into parental immune cell subsets and calculation of subset frequencies accordingly.

WS.A2.03.02

Aging affects the frequency, functions and antibody repertoire of human B-1 cells

A. M. Hernández-Vázquez, A. Rodríguez-Zurbarán, T. Quach, T. J. Hopkins, T. L. Rothstein; 1

1Center for Molecular Immunology, Havana, Cuba, 2Feinstein Institute for Medical Research, New York, United States, 3Center for Immunobiology of the Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, United States.

Introduction: Aging decreases the efficiency of the immune system, which has been associated with an increased incidence of infections, autoimmune diseases and cancer. Human naive and memory B cells suffer significant quantitative and qualitative changes in the elderly. However, human B-1 cells, which play critical housekeeping and anti-microbial defensive roles, have not been studied. In the present work we analyzed aged naïve and memory (CD19+CD27+CD38-CD24+) B-1 cells.

Materials and the Methods: The frequency of human B-1 cells was studied by flow cytometry. The capacity of these cells to produce IgM was detected by ELISPOT and their repertoire and genes related with Ig secretion were studied by single cell PCR.

Results: Our results show that the percentage of B-1 cells, but also their capacity to spontaneously secrete IgM decrease with age. Expression levels of Xbp1 and Blimp1, associated with Ig-secreting phenotype, were significantly lower in elderly donors compared to young. Furthermore, CD21+CD19+CD27+CD38-CD24+ B-1 cells showed a reduction of the antibody repertoire variability in comparison with young individuals. It was interesting to observe differences in the usage of certain VH and DH families among young and elderly individuals. We also proved that B-1 cells are involved in the secretion of antibodies against NeuGcGM3 ganglioside, a tumor associated antigen, and this specificity also decreases with age.

Conclusions: These results show changes in the frequency and function of human B-1 cells with aging, which could affect their protective and homeostatic functions.
WS.A2.03.03

T cell immunity does not age in a long-lived rodent species

M. Izraelson1, T. Nakonechnaya1,2, A. Daydov1, M. Drionova1, D. Miskevitch1, I. Mamedov1, L. Barbashova1, M. Shugay1,4, D. Bolotin1, D. Staroverov1, E. Kondratyuk1, S. Lukyanov1, I. Shams1, O. Bentin2, D. Chudakov2,3

1Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation, 2Nichyev Novgorod State Medical Academy, Nichyev Novgorod, Russian Federation, 3PIRGov Russian National Research Medical University, Moscow, Russian Federation, 4Central European Institute of Technology, Brno, Czech Republic, 1Institute of Evolution & Developmental Biology and Environmental Biology at University of Hafija, Hafija, Israel, 2Center for Data-Intensive Biomedicine and Biotechnology, Skolkovo Institute of Science and Technology, Moscow, Russian Federation, 3Institute of Systematics and Ecology of Animals SB RAS, Novosibirsk, Russian Federation.

The T cell receptor (TCR) diversity of naive T lymphocytes represents a precious collection of keys from which antigen-specific variants are selected, conferring protection for the host against new challenges. Here we analyzed peripheral TCR repertoires from healthy humans, mice, and blind mole-rats (Spalax sp.)—long-lived, cancer-resistant rodents. We report that Spolax T cell diversity remains stable even for animals that reach extreme old age (15-17 years), in striking contrast to mice and humans, for whom immunosenescence is associated with the accumulation of large numbers of memory clones. This discovery reveals a distinctive strategy for T cell immunity organization that potentially underlies the extraordinary longevity of Spalax, and encourages a re-evaluation of the contribution of immunosenescence to life span in mammals. The work was supported by the Russian Science Foundation project No 16-15-00149 (to BOV). Mice samples were preparation was supported by grant of the Ministry of Education and Science of the Russian Federation.

WS.A2.03.04

Antigen-specific immunity is inhibited in the skin of older people by p38 MAPK-driven inflammatory monocytes

E. S. Chambers1, M. Vukmanovic-Stejic1, H. Trohaci1, A. Appios1, V. Biraui2, M. Nousaradehi1, N. Mabbott2, M. Rustin2, A. Akbar1

1University College London, London, United Kingdom, 2The Francis Crick Institute, London, United Kingdom, 3The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom, 4Royal Free Hospital, London, United Kingdom.

Decline in immunity with age is often associated with an increase in low grade chronic inflammation, termed ‘inflammageing’. We have shown that there is a large proinflammatory response to a saline injection in the skin of >65 years old people which is not observed in the young (<40 years). Bioinformatic and immunohistochemical analysis demonstrated that the inflammatory saline response is driven by recruitment of inflammatory monocytes (CD14+CCR2+) and a p38-MAPK inflammatory gene signature. Importantly this inflammatory response to saline is negatively correlated with an antigen specific response in the skin to VZV.

Methods: A Th17 differentiation-driven expansion assay with a Cd4+ T cells from older individuals was used to screen a library of epigenetic inhibitors. Th17 and IL-17A cytokine production were measured using flow cytometry and ELISA respectively. siRNA gene knockdown was used for target validation, RNA-seq and qPCR were used to investigate target validation.

Results: By the unbiased screen of epigenetic inhibitors using CD4+ T cells from AS patients, we aim to reveal novel mechanisms of epigenetic regulation in Th17 cells and identify potential therapeutic targets for Th17 pathogenicity.

Introduction: A Th17 differentiation-driven expansion assay with CD4+ T cells from older individuals was used to screen a library of epigenetic inhibitors. Th17 and IL-17A cytokine production were measured using flow cytometry and ELISA respectively. siRNA gene knockdown was used for target validation, RNA-seq and qPCR were used to investigate downstream pathways.

Conclusions: We have identified D1, a p38-MAPK inhibitor, as a potent and selective epigenetic suppressor for Th17 cells. BRPF1 and BRPF2 regulate Th17 response through distinct mechanisms.

WS.A2.04

Evolution of immune responses in health and disease

WS.A2.04.01

Hematopoietic progenitors from old humans are metabolically active but present evidence of cellular senescence and pyroptosis

T. Fol1, V. Fabre Mersseman2, T. Yamamoto3, J. Boddart1, D. Sauce4, V. Appay4

1INSERM CIRM, Paris, France, 2National Institutes of Biomedical Innovation, Osaka, Japan, 3AP-HP Service de Gériatrie, Paris, France, 4Kumamoto University, IRCMS, Kumamoto, Japan.

The maintenance of effective immunity over time is dependent on the capacity of hematopoietic stem cells (HSC) to sustain the pool of immunocompetent mature cells. Decline of immune function in humans with old age may stem from HSCs, including senescence and impaired self-renewal potential, and impairs stemness, as suggested in murine models. To get further insights into aging related alteration of hematopoiesis, we performed a comprehensive study, including phenotypic, transcriptomic and functional assessments, of blood hematopoietic progenitor cells (HPC) from elderly humans (i.e. >75 years old). In the elderly, HPC present active oxidative phosphorylation and are pressed to enter cell cycling. However, p53-p21 and p15 cell senescence pathways, associated with telomerase activity deficiency, are engaged, thus limiting cell cycling. Moreover, survival of HPC is impacted by pyroptosis, an inflammatory form of programmed cell death. Loss, telomerase activity deficiency and telomere length attrition of old HPC may be passed on progeny cells such as naïve T lymphocytes, highlighting further the poor lymphopoietic potential and capacity to induce T-cell responses of the elderly. This pre-senescence profile is characteristic of the multiple intrinsic and extrinsic factors affecting HPC in old individuals and represents a major obstacle in terms of immune reconstitution and efficacy with advanced age.

WS.A2.04.02

IMMUNOBIOMGRAM: a new immunological tool to personalize immunosuppressive therapy in kidney transplant recipients

M. Di Scala1, J. Portolés1, C. Jiménez1, D. Janeve1, E. González1, B. Sánchez Sobrino1, M. López Oliver1, J. Richter1, A. Ortega1, J. Pascual1, J. Portela1

1IMPOPE Scientific SL, Madrid, Spain, 2Hospital Universitario Puerta de Hierro, Neurology Department, Madrid, Spain, 3Hospital La Paz, Nephrology Department, Madrid, Spain, 4Hospital del Mar, Nephrology Department and Kidney Transplantation, Barcelona, Spain.

INTRODUCTION: Transplant rejection is one of the biggest limitations in kidney transplantation(.KT). It remains essential to control the immune-mediated tissue-specific destruction using immunosuppressive drugs (IMM) that limit immune system hyperactivation and prevent the allograft loss. IMM's regimens are stabilized based on standard clinical guidelines and empirically.

44

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
WORKSHOPS

BIOHOPe is developing a blood-based Precision Medicine test for KT. It offers a personalized comparative evaluation of patient sensitivity to a panel of IMiDs most commonly used. This functional pharmacodynamic and monitoring kit is named Immunobiogram (IMBiG), an immunocassette based on the concept of the antibiotic.

METHODS: IMBiG is a 3D-cell culture of PBMCs included in a hydrogel capable of spontaneous generation of IMiDs concentration gradient due to a passive-diffusion process. An indicator of cell-proliferation/viability reveals the capacity of IMiDs-gradient to inhibit the activation cells state.

RESULTS: IMBiG was evaluated in BH-pilot study performed in two major Hospitals in Spain. It included 70 patients belonging to three immunological risk-categories (low-risk, standard, high-risk patients).

The resulting profiles were used to ascertain the sensitivity of each patient to each IMiD and to establish a panel of IMiDs recommendation for the clinical management. Significantly associated low-sensitivity patterns have been observed in patients who present worse clinical evolution.

CONCLUSIONS: IMBiG provides an automated method to quantitatively measure the response of a patient to IMiDs that will aid clinicians in the determination of the optimal combination/position of IMiD-immune-modulator drugs and opens the possibility to make the necessary adjustments in immunosuppressive therapy to avoid chronic rejection and reduce side-effects of IMiDs.

WS.A2.04.03
Particular neutrophils mediate inflammatory-driven event of airway透过 through NET release
C. Radermecker1, C. Sabatei, S. Johnston2, M. Toussaint1, F. Bureau1, T. Marichal1
1GIGA-R, Cellular and Molecular Immunology, Sant-Tilman, Belgium, 2Imperial College, London, United Kingdom.

Environmental changes are responsible for the dramatic rise in the prevalence of allergic asthma worldwide. Decreased exposure to microbial products such as lipopolysaccharide (LPS) and respiratory viral infections represent two major risk factors for asthma, yet the mechanisms linking such conditions and host allergic susceptibility remain unclear. First, we will discuss a recent study showing that NETS are increased in patients experiencing respiratory viral infections. Second, we will present preliminary results from a study on patients exposed to low LPS doses in vitro and mice exposed to low LPS doses displayed the characteristics of asthma following sensitization and challenge to house dust mite (HDM). Then, using single-cell RNA sequencing, we found that pro-allergic environments (low LPS doses and respiratory virus) induced the recruitment into the lungs of CCR4+CD49d+LAMP1−1 α-Neutrophils releasing neutrophil extracellular traps (NETs). The role of NETs in asthma onset was then demonstrated using three NET-losers in our two models. Infected or low LPS doses exposed mice exhibited significant decrease of all asthma features when treated with NET-losers inhibitors compared to non-treated mice. Finally, to address how NETs promote the development of a Th2 immune response, we analysed by flow cytometry the distinct subpopulations of lung dendritic cells (DCs) in our models. We observed, during the NETs release phase, a recruitment of monocytic-derived DCs responsible for allergic sensitization. This recruitment was abrogated when NETs were inhibited. In conclusion, our study reveals how apparently unrelated environmental risk factors commonly shape immune responses, by recruiting particular neutrophils which release NETs, to promote asthma.

WS.A2.04.04
Alpha-Gal bond to lipids, but not to proteins, is able to cross the intestinal epithelium and might thus cause delayed allergic symptoms in meat allergic patients
P. Román-Carrasco1, B. Lieder1, V. Somoza1, M. Ponce1, S. Zépfalvi2, D. Martír1, W. Hemmer1, J. Swoboda1
1Molecular Biotechnology Section, University of Applied Sciences, Vienna, Austria, 2Department of Physiological Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria.

The oligosaccharide galactose-α-1,3-galactose (α-Gal), present on mammalian proteins and lipids, causes an unusual delayed allergic reaction 3 to 6 hours after ingestion of meat from animals with an individual with α-Gal bond. We analyzed the α-Gal-specific activated NETs and NETs to cross the intestinal epithelium. For this, extracts of proteins and lipids from cooked beef were prepared, subjected to in vitro simulated digestions and added to Caco-2 cells grown on permeable inserts. The presence of α-Gal was investigated in the basolateral medium by immunoblotting, thin-layer chromatography (TLC) with immunostaining and ELISA and its allergenic activity was analyzed in a basophil activation test. Antibody binding of α-Gal proteins on the apical side of Caco-2 cells, α-Gal containing peptides were not detected. The Caco-2 monolayer did not activate basophils from a α-Gal allergic patient. Instead, when Caco-2 were incubated with lipids extracted from beef, α-Gal was detected in the basolateral medium. Furthermore, these α-Gal could be activated by the basophils of an α-Gal allergic patient in a dose-dependent manner. We thus showed that only α-Gal carried on lipids, but not on proteins is able to cross the intestinal epithelium and trigger an allergic reaction. The slower activation process of α-Gal conjugated lipids might explain the delay in the appearance of symptoms since it takes longer before the tissues is able to cross a lipid barrier than for proteins to reach the circulation.

WS.A2.04.05
Cytoplast: A workflow for flow and mass cytometry data to discover group-related immune signatures
G. Beyrend, K. Stam, T. Holst, R. Aens
Leiden University Medical Center, LEIDEN, Netherlands.

Multi-parametric flow and mass cytometry allows exceptional high-resolution exploration of the cellular composition of the immune system. A large panel of tools have been developed to analyze the high-dimensional landscape of the data generated. Among them, Cytoscape, incorporating the HSNE algorithms, is highly suitable for multi-parametric cytometry analysis. However, this software focuses on visual exploration and does not provide means to quantify group-specific cell clusters or correlations with clinical outcomes. Here, we introduce an R-based workflow, called CytoPlast, for downstream analysis of Cytosplote-processed cytometry data sets. CytoPlast is generating visual representations to identify group-related immune cell clusters and to study the correlation of any clinical variable with the immune system composition. We apply our workflow on two previously published data sets, paired and non-paired, based on the T-cell receptor repertoire immuno-phenotyping study, which decreased upon allogeneic tumor-binding IgG. Thus, our bioinformatic tool offers an automated time-efficient approach for comprehensive multi-parametric cytometry analysis to reveal group-related immune signatures.

WS.A2.04.06
A novel algorithm for analysis of the evolution of B-cell receptors repertoire using high-throughput sequencing data
A. S. Obratzsova1, M. Shugay1,2,3,4, D. M. Chudakov1,2,3,4
1Skolkovo Institute of Science and Technology, Moscow, Russian Federation, 2Institute of Bioorganic Chemistry (RAS), Moscow, Russian Federation, 3Pirogov Russian National Research Medical University, Moscow, Russian Federation, 4Privolzhsky Research Medical University (PIMU), Moscow, Russian Federation, 5Central European Institute of Technology (CEITEC), Prague, Czech Republic.

One of the mechanisms that is critical for forming an efficient immune response is the affinity maturation of B-cells, a process during which somatic hypermutations (SHMs) are introduced into the lg sequence followed by subsequent rounds of antigen-driven selection in germinal centers of lymph nodes. Recent advances in high-throughput sequencing techniques empowered a comprehensive study of this phenomenon, but some technical hurdles still complicate the analysis of B-cell receptor repertoire. The present work is devoted to the solution of two problems. First, the background noise of PCR and sequencing errors that can interfere with SHM analysis can be eliminated using the molecular tagging technique and an extension of our previously published algorithm [1] to full-length Ig-sequences. The second difficulty lies in the identification of SHMs that fall into the complementarity-determining region 3 (CDR3) of the lg sequence that doesn’t have any germline reference sequence. To solve the latter problem, we have developed a novel algorithm that can accurately identify and cluster CDR3 sequences that are far more similar than can be expected from random sampling of CDR3 repertoire. This approach allows tagging technique and an extension of our previously published algorithm [1] to full-length Ig sequences. The second difficulty lies in the identification of SHMs that fall into the complementarity-determining region 3 (CDR3) of the lg sequence that doesn’t have any germline reference sequence. To solve the latter problem, we have developed a novel algorithm that can accurately identify and cluster CDR3 sequences that are far more similar than can be expected from random sampling of CDR3 repertoire. This approach allows tagging technique and an extension of our previously published algorithm [1] to full-length Ig sequences.

WS.A3.01.01
Immunobiomarkers in autoimmunity and beyond
WS.A3.01.01
Dissecting IL-12p70 response variability in health and disease
C. Passemé1, B. Chardot1, A. Libere1, A. Bisaux1, B. Pasecka1, E. Nemes1, T. Scriba2, L. Quintana-Murci3, S. Pol, M. L. Albert1, D. Dufour3
1Institut Pasteur, Paris, France, 2SKAT, Cape Town, South Africa, 3GeneTeck Inc, San Francisco, USA.

Cytokines are essential regulators of immune responses and coordinate the response against pathogens. Therefore, they hold great potential as targets for new diagnostic and therapeutic strategies. IL-12p70 is a key heterodimeric molecules for driving Th1 immune responses. Despite its role in combating infection we have observed highly variable IL-12p70 responses in healthy individuals, as well as in patients infected with tuberculosis (TB) and chronic hepatitis C (HCV). We hypothesize that dissecting this variance in a healthy population will provide new insights into disease pathogenesis. Following LPS stimulation of whole blood from 1,000 donors of the Milieu Interieur cohort, 28% of healthy donors exhibited IL-12p70 responses in healthy individuals, as well as in patients infected with tuberculosis (TB) and chronic hepatitis C (HCV). We hypothesize that dissecting this variance in a healthy population will provide new insights into disease pathogenesis. Following LPS stimulation of whole blood from 1,000 donors of the Milieu Interieur cohort, 28% of healthy donors exhibited IL-12p70 responses.
Utilizing the Milieu Interieur cohort we have further investigated the factors behind this variability. Having identified by flow cytometry that monocytes and dendritic cells are the most enriched cell type in this liquid biopsy, we performed a round robin study to evaluate the variability in the measurement of immune cells. In contrast to gene expression analysis, revealed significant IFNγ response differences between the two phenotypes under LPS stimulation, and protein Quantitative Trait Loci analysis revealed a genetic association with IL-12p70 responses. These results will allow to investigate the intracellular mechanisms by blocking specific pathways and to restore them in chronically infected HCV or HB patients.

WS.A3.01.02

Background: Rituximab (RTX) has shown clinical efficacy in autoimmune and immune diseases but to up to 40 % of RTX treated rheumatoid arthritis (RA) patients are poor responders (ref 1) and the commonly used RA biomarkers (RF/ACPA) are poor predictors for therapy response. Methods: Screening of RA sera was conducted on 37.830 unique human protein macroarrays (http://www.engine-gmbh.de) with sera taken before and 24 weeks after treatment. Autoantibody response of different immunoglobulin classes IgG, IgA, and IgM was recorded and bioinformatically evaluated. Response was determined according to DAS28 criteria. Results: In the cohort of 26 patients 1292 different autoantigens (IgG/IgA/IgM) were detected. Using protein array we investigated clusters of autoantigens that disappeared or developed during RTX treatment of RA patients. Post treatment developing responses against new blood, we confirmed that cellular effectors were responsible for new autoantigenic patterns before and after 6 month after RTX treatment were patient specific. RTX reduced the repertoire of autoantibodies after 24 weeks of treatment in the tested RA patient cohort on average by 60%. RA patients which do not respond are generating on average 63% new autoantibodies. In good responders to RTX only 5,5% (+/-3%) new autoantibodies can be detected. The IgG and IgA autoantibody repertoire in the serum after 24 weeks of RTX treatment is reduced (IgA: 41%, IgG: 31%) in good responders whereas it is increased (IgA: 1,3%; IgG: 24%) in non responders to RTX. Conclusions: Non responders to RTX their change antibody repertoire directed against new but patient specific antigens. References: 1.AnnRheumDis. 2005 Feb;64(2):146-32.

WS.A3.01.03

Imbalance of naive and memory T cells in peripheral lymphocyte subpopulations at onset of type 1 diabetes A. Teniente-Serra1, E. Pizarro2, C. Esteve-Cals1, M. Julian1, M. Fernández, E. Martínez-Cáceres2; 1Germans Trias i Pujol University Hospital, Badalona, Spain, 2Hospital de Marató, Marató, Spain, 1Germans Trias i Pujol Research Institute (IGTP), Campus Can Ruti, Badalona, Spain.

Introduction: Type 1 diabetes (T1D) is an autoimmune disorder characterized by destruction of pancreatic beta cells resulting in insulin dependency. Changes in T and B cell subpopulations in peripheral blood of T1D patients have been described previously but a comprehensive high-parametric flow cytometric analysis is still lacking.

Aim: To identify changes in peripheral blood T- and B-cell compartments in patients at onset of T1D.

Material and methods: CD4+ and CD8+ T cells (including naive, central memory, effector memory and terminally differentiated effector (TEMRA), Th1 and Th2) and B cells subsets (naive, unswitched memory, switch memory and transitional B cells) were analyzed in peripheral blood of T1D patients at onset (n=26) and healthy donors (n=40) using multiparametric flow cytometry.

Results: A decrease in the percentage of early and late effector memory CD4+ and CD8+ T cells (TCD4+: p=0.001 and p=0.001, TCDB+: p=0.046 and p=0.001, TEMRA CD4+ and CD8+ cells (p=0.003 and p=0.004, respectively) was found. In contrast, the percentage of naive CD4+ T cells (p=0.010), and percentage and absolute counts of naive CD8+ T cells (p=0.001 and p=0.001) were increased in peripheral blood of T1D patients compared with HD. Moreover, an increase in percentage of total B cells and transitional B cells was observed in patients compared with HD (p=0.04 and p=0.03, respectively). No changes were observed neither in naive CD4+ nor in naive CD8+ T cells.

Conclusion: The observed changes in the percentage and/or absolute number of lymphocyte subpopulations support that effector cells migrate to the pancreas participating in the autoimmune response.

WS.A3.01.04

Glycolytic T cell metabolism drives faster disease progression in progressive multiple sclerosis patients S. De Biasi1, E. Bianchini, M. Nasi, L. Giebils1, S. Perocchi1, D. Lo Tartaro1, A. Simone, D. Ferraro1, F. Vitetta1, P. Solà, A. Cossarizza1, M. Pinti1; UNIVERSITY OF MODENA AND REGGIO EMINA, MODENA, Italy.

Introduction. Inflammation and neurodegeneration sustain disease progression in both primary progressive (PP) and secondary progressive (SP) form of multiple sclerosis (MS). PP and SP forms present different symptoms, but the inflammatory status and the neurodegenerative process are indistinguishable. Differences in T24 T cell biology identify PP patients having different rates of progression, emphasizing an association between the systemic immune activation and disease progression. However, the mechanisms responsible of such impairment are still unknown.

Aim. T cell activation is accompanied by a switch from a metabolism mainly based upon mitochondrial respiration to a metabolism where glycolytic flux is prevalent. Hence, we investigated the metabolic changes and mitochondria (mt) functionality of T cell subpopulations in 46 progressive MS patients, to clarify if PP form is part of the disease spectrum, or a distinct entity.

Results. When compared to SP patients, T cells from PP displayed a senescent phenotype (low proliferation, increase of terminally differentiated/exhausted cells), lower mt mass, membrane potential and respiration, a more marked down-regulation of transcription factors supporting respiration.

This is counterbalanced by higher mTOR activity and higher expression of glycolytic-supporting genes. Higher levels of lactate were found in plasma of PP patients. Conclusion. SP and PP patients displayed differences from the phenotypic and metabolic point of view. These differences, driven by deficit or abnormalities in the metabolism of immune cells, can contribute to neurodegeneration and chronic level of inflammation in PP and SP patients, determining a faster disease progression in PP.

WS.A3.01.05

Measurement of serum infliximab levels and detection of free and bound anti-infliximab antibodies in patients with rheumatoid arthritis C. Hermannrud1, M. Ryner1, R. Pullerits1, K. Hambardzumyan1, N. Vivar Pomian1, R. Marits1, I. Gjerstson1, S. Sævarsdottir1, A. Fagrell-Hahn1; 1Clinical Neuroimmunology, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; 2Department of Rheumatology and Inflammation Research, Sahlgrensk Teaching University, Karolinska Institute, Gothenburg, Gothenburg, Sweden, 3Department of Clinical Immunology, Sahlgrensk University Hospital, Gothenburg, Sweden, 4Rheumatology Unit, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden, 5Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden.

Introduction: Tumor necrosis factor-α (TNF-α) inhibitors are used to treat symptoms of rheumatoid arthritis (RA). Low drug levels correlate well with the presence of anti-drug antibodies (ADA) and are likely to result in a poor clinical outcome. Levels of free TNF-α inhibitor and ADA are detected with an in-house validated ELISA used in clinical routine at Karolinska University Hospital. However, false negative results can occur due to the formation of drug/ADA immune complexes and that samples with a drug level >0.5 µg/ml should not be screened for ADA with ELISA because of the assay’s low drug tolerance. Methods: Drug levels and ADA were studied with three methods; free ADA (ELISA), neutralizing ADA (bioassy, iL-17), and total ADA (precipitation and acid dissociation assay, PandA). Three RA cohorts were included; SWEFOT (prospective, n=101) Karolinska University Hospital, and REALIFE with patients from Karolinska University Hospital, (cross-sectional, n=272) and Sahlgrenska University Hospital (prospective, n=42). Results: The majority of patients (SWEFOT 46%; REALIFE 70%) had suboptimal TNF-α inhibitor serum concentration (<3 µg/ml), and around 24% had an optimal drug concentration (3-7 µg/ml). A high drug inhibitor level correlated with neutralizing ADA, with ADA, of which 66% were neutralizing. Moreover, PandA method detected free and bound ADA reactivity in 24% of tested serum samples that had detectable TNF-α inhibitor levels (0.2-7 µg/ml). Conclusion: There was a clear difference in the drug level between the clinical trial cohort and the real-life situation, showing that expected ADA prevalence might need to be adjusted in a routine clinical setting.

WS.A3.01.06

Imaging flow cytometry enhances the detection of small particles and rare events enabling emerging applications in immunology and oncology P. Rhein1, A. Goergen2, B. Giebel3, S. Groenefeld-Krentz4, C. Eckert5; 1Merck KGaA, Darmstadt, Germany; 2Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, 3Institute of Transfusion Medicine, University Hospital Essen, Essen, Germany, 4Pediatric Oncology/Hematology, Charité-Universitätsmedizin, Berlin, Germany.

Detection of small particles and rare events by flow cytometry is often hampered by the limited amount of information that can be gathered from light scatter signals and fluorescence. Small particles like extracellular vesicles (EVs) have recently gained increased interest as they are physiologically and diagnostically relevant. The small but variable size and abundance made analysis of single EV often difficult on traditional flow cytometers. For rare events detection discrimination of relevant events and artifacts is absolutely mandatory and requires complex and challenging experimental design for traditional flow cytometers. One example are circulating tumor cells (CTCs), released into the bloodstream from primary and metastatic cancers and tumor cells persisting with they have in them remaining (minimal residual disease, MRD) that have important prognostic and therapeutic implications and are valuable tools for understanding tumor biology. The ImageStream® is a multispectral imaging flow cytometer that helps to overcome these obstacles by combining the statistical power of flow cytometry with imaging content of microscopy in one system. We are now able to characterize EVs including exosomes (70 nm - 160 nm in diameter), microvesicles and open different gates for the detection of CTCs by preventing the background of the false positive signals of EVs CD45+ events as leukocytes and the undercounting of CTC doubles as single cells. And we demonstrate the feasibility of fluorescence in situ hybridization (FISH) in flow which can contribute to significantly improve the detection and functional analysis of biological rare events.
WORKSHOPS

WS.A3.02 Biomarkers of adaptive immunity

WS.A3.02.01
Rapid identification and isolation of functional antigen-specific CD8+ T cells by staining of activated integrins


1University of Tübingen, Tübingen, Germany, 2University of Copenhagen, Copenhagen, Denmark, 3University of Lubeck, Lubeck, Germany.

Immediate changes in the conformation and clustering of β1-integrins upon T-cell receptor stimulation is critical for the strong adhesion of antigen-specific T cells to their targets and the downstream execution of T-cell effector functions. Integrin activation may therefore be used for the rapid identification of functional T cells. We present a novel, simple, and sensitive flow cytometry-based assay to assess antigen-specific T cells using fluorescent intercellular adhesion molecule (ICAM)-1 multimers that specifically bind to activated β1-integrins. The method is compatible with surface and intracellular staining; it is applicable for monitoring of a broad range of virus-, tumor- and vaccine-specific CD8+ T cells, and for isolating viable antigen-reacting cells. ICAM-1 binding correlates with peptide-MHC multimer binding, but, notably, it identifies the fraction of antigen-specific CD8+ T cells with immediate and high functional capability, expressing high levels of cytokotic markers and cytokines. Compared to the currently available methods, staining of activated β1-integrins presents the unique advantage of requiring activation times of only several minutes, therefore delivering functional information nearly reflecting the in vivo situation. Hence, the ICAM-1 assay is most suitable for rapid and precise monitoring of antigen-specific functional T-cell responses including for patient samples in various clinical settings.

WS.A3.02.02
Epigenetic signatures of human T helper cell subsets

A. Ntalii, S. Kumar, L. Maggi, F. Annunziato, C. Zinner, M. Beckstette, L. Groc, S. Flossi, J. Huehn

1Department of Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany, 2Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal, 3Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy, 4Genomatica Software GmbH, Muenchen, Germany.

Naive CD4+ T cells are highly plastic cells that - upon activation - can differentiate into various T helper (Th) cell fates characterized by the expression of specific transcription factors and effector cytokines. Their expression can be stabilized by epigenetic mechanisms including DNA methylation. So far, our knowledge about the link between DNA methylation and T-cell helper differentiation processes is fragmentary. After the identification of an epigenetic Th17 signature suitable for the characterization of murine cells, we wanted to extend this knowledge to the human epigenome by performing a whole-genome bisulfit sequencing analysis of ex vivo isolated human naive T cells and selected clones of Th1, Th2, Th-cells. The comparison of the different methylomes allowed for the detection of differentially methylated regions (DMRs) mainly located within introns, followed by promoter regions and exons. In accordance with literature, several DMRs were identified within lineage-specific loci like IFNG, TBX21, IL17A and RORC2. Additionally, we identified demethylated and closed promoters or plasmidic structures to study their transcriptional readout in primary human CD4+ T cells revealed that DMRs within SLAMF8 and SR5F7 mediate transcriptional activity in a methylation-dependent fashion. In addition, we performed expression studies using ex vivo isolated naive Th1, Th2 and Th17 helper cells to identify transcription factors that participate in transcriptional regulation via the DMRs. Thereby, we might be able to define lineage-specific regulatory modules involved in human T helper cell differentiation.

WS.A3.02.03
The cold shock protein YB-1 (Y-box binding protein 1) promotes survival of CD4+ T-lymphocytes

S. Meltdorf, S. Gieseler-Hailbach, J. Handschuh, M. Pierau, A. Ared, P. R. Mertens, U. Thomas, M. C. Brunner-Weinzierl

1Department of Experimental Paediatrics, Magdeburg, Germany, 2Leibniz Institute for Neurobiology, Magdeburg, Germany, 3Department of Nephrology and Hypertension, Diabetes and Endocrinology, Magdeburg, Germany, 4Department of Neurochemistry and Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg, Germany.

The cold shock protein YB-1 is highly expressed in tumours, such as breast cancer, and associated with hyper proliferation and resistance against apoptosis. Enhanced YB-1 expression at the transcription and nuclear protein levels have been shown to correlate with poor prognosis and resistance to chemotherapy for tumour patients. However, the role of YB-1 in T-lymphocytes is not understood so far. Human CD4+ T-lymphocytes isolated from PBMCs were stimulated with anti-CD3/anti-CD28 coupled beads. YB-1-signalization was manipulated by overexpression of YB-1 through lentiviral transduction of FUW-EXP-constructs or reduction of YB-1 expression using specific YB-1-shRNA. Apoptosis measurement was carried out six to eight days after initial stimulation and analysed. Expression of pro- and anti-apoptotic molecules was monitored by flow cytometry, western blot, and real-time PCR. Transduction of GFP-YB-1-constructs and mutant variants thereof into primary human CD4+ T-cells yielded around 40% GFP positive cells. We observed that YB-1 overexpression enhanced survival by 60% compared to the control. Additionally, the percentage of surviving cells was increased with specific inhibitors (QVD) for apoptosis in ex vivo-transduced cells but not in YB-1 overexpressing cells. Furthermore, forced reductions of YB-1 with YB-1-shRNA lead to an reduced survival in T lymphocytes. A substantial reduction in the mRNA and protein levels of anti-apoptotic molecule Bcl-xl following YB-1 knock-down clearly indicated the impact of YB-1 in regulating the T cell apoptosis. Thus, YB-1 tightly controls and promotes survival in T cells.

WS.A3.02.04
Tissue resident memory T cells as a progressive multifocal leukoencephalopathy quick biomarker


1University of Malaga, Malaga, Spain, 2Hospital Gregorio Marañón, Madrid, Spain, 3Hospital La Paz, Madrid, Spain.

Introduction: Progressive multifocal leukoencephalopathy (PML) is a serious side effect associated with immune system modifying drugs in multiple sclerosis (MS). PML is caused by the opportunistic infection by John Cunningham virus and its early diagnosis is crucial for patient's survival. Tissue resident memory T cells (T RM) are important during viral infection, and they can be detected in cerebrospinal fluid (CSF) by flow cytometry in only one hour. We aimed to explore if PML patients show an increase in T RM cells and, in addition, to explore other CD4 and CD8 T cell subsets.

Patients and methods: We included 66 MS patients: three suffered PML (PML+), and 83 did not (PML-). CSF T cell subsets were explored by flow cytometry in a FACSCanlo III cytometer. Results were expressed as percentage of CD45 mononuclear cells. Mann-Whitney U test was applied for comparison between groups.

Results: PML− showed lower CD4 (p=0.0062), naïve CD4 (p=0.0111) and central memory CD4 (p=0.0440) compared to PML-. By contrast, in PML+ we observed a higher percentage of naïve CD4+ T cells, naïve CD8+ T cells, and TCM CD8+ T cells (p=0.0032). A substantial reduction in the mRNA and protein levels of anti-apoptotic molecule Bcl-xl following YB-1 knock-down clearly indicated the impact of YB-1 in regulating the T cell apoptosis. Thus, YB-1 tightly controls and promotes survival in T cells.

Conclusions: Although these findings should be validated in a larger cohort, our data show that PML induces a significant change in CSF T cell subsets of MS patients and strongly suggest that T RM detection could be a good tool for an early PML diagnosis.

WS.A3.02.05
T3SS components as the biomarkers of humoral immune response elicited by live bacterial vaccine in humans


1Federal Research Center for Virology and Microbiology, Branch in Saratov, Saratov, Russian Federation, 2Saratov State Agricultar University named after N.I. Vavilov, Saratov, Russian Federation, 3Saratov State Medical University named after V. Razumovsky, Saratov, Russian Federation, 4Saratov State National Research University, Saratov, Russian Federation.

Type III Secretion System (T3SS), a system of proteins known as a powerful bacterial tool for subverting host immune response by Gram-negative pathogens. T3SS components as the key virulence factors are an attractive target for both vaccine and immunological markers development. In this study, using immunoblot technique and highly pure recombinant antigens, we investigated the potential of T3SS components, namely LcrV, YopM and YopE, to serve as the markers of humoral immune response in humans multiply vaccinated with live plague vaccine LPV, an attenuated K. pestis EV strain line NIE6 possessing T3SS (n = 34). Sera from healthy naïve volunteers (n = 17) were used as a control. Anti-YopE antibodies, but not anti-YopM or anti-LcrV, were highly specific for the vaccinated group (p<0.05). Interestingly, humoral response to both YopE and YopM was likely short-lasting since it correlated negatively with the post immunization period (p<0.05). There was an inverse association between the number of LPV injections and positive responses.
abstracts of the workshops

WS.A3.02.06
Skin-homing human CD8+ T cells preferentially express GPI-anchored peptidase inhibitor 16 (PI16), an inhibitor of cathepsin K
1Semmelweis University, Dept. of Genetics, Cell and Immunology, Budapest, Hungary; 2Hungarian Academy of Sciences - Semmelweis University Immunoproteogenomics Extracellular Vesicle Research Group, Budapest, Hungary; 3Office for Research Groups Attached to Universities and Other Institutions of the Hungarian Academy of Sciences, Budapest, Hungary; 4Department of Hematology and Stem Cell Transplantation of the St. Istvan and Saint Loszló Hospital, Budapest, Hungary; 53rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary;
This study sought to identify novel markers of skin- and gut-homing CD8+ T cells by analyzing them in acute graft versus host disease (aGVHD). Typically involving CD8+ T-cell mediated organ damage of the skin and gut.
Patients undergoing allogeneic hematopoietic stem-cell transplantation were assigned to groups developing cutaneous aGVHD, gastrointestinal aGVHD, both, or none, and their sorted skin-homing (CD8+/CLA+), gut-homing (CD8+/Integrinβ7+), and reference (CD8+/CLA+/Integrinβ7−) T cells were compared. Microarray analysis, Q-PCR and flow cytometry disclosed increased expression of peptidase inhibitor 16 (PI16) in skin-homing CD8+ T cells. PI16 was expressed by CD8+ T cells regardless of the organ(s) affected by aGVHD, aGVHD as such, and remained associated to skin-homing T cells in healthy blood donors, too. PI16 was not observed on CLA+ leukocytes other than T cells, and was restricted to the non-naïve CD45RO+ compartment. Induction of PI16 expression was independent of vitamin D3, remained unaffected by retinoic acid, or by co-culture with human skin and intestinal organoids. PI16 was confined to the plasma membrane, appeared GPI-anchored, and became lost upon re-stimulation of circulating skin-homing T cells. Loss of PI16 occurred by rapid downregulation of PI16 transcription, not by PLC- or ACE-mediated shedding, or by recycling from the plasma membrane. Inhibitor screening and pull-down experiments confirmed that PI16 has low affinity, if any, to most skin proteases, but inhibits cathepsin-K.
These data demonstrate robust PI16 expression in skin-homing CD8+ T cells, and raise the possibility that PI16 may inhibit an inflammatory skin protease until cutaneous CD8+ T-cell activation.

WS.A3.03 Immune markers in malignancies

WS.A3.03.01
NSCLC patients not responding to nivolumab show lowered frequency of co-stimulatory receptor-deficient CD8 T cells
Erasmus MC Cancer Institute, Rotterdam, Netherlands;
Checkpoint inhibitors have become standard care of treatment for non-small cell lung cancer (NSCLC). As only a limited fraction of patients experience durable clinical benefit, there is an urgent need to identify patients with early progressive disease. In the current discovery study, we have analyzed peripheral blood samples of 71 NSCLC patients treated with 2nd line nivolumab prior and throughout therapy with multiplex flow cytometry enumerating 20 immune cell subsets, and assessing frequencies of T cells using over 300 combinations of markers. Best overall response was assessed according to RECIST within 90 days from start of treatment. We discovered that patients with progressive and stable disease (PD and SD) displayed on average a 2-fold decrease in numbers of CD8 T cells prior to and throughout therapy, while patients showing partial response (PR) showed levels similar to those of healthy individuals with about 560 cells/μl. Analysis of T cell subsets expressing variable numbers of co-stimulatory or co-inhibitory receptors revealed that this reduction was accompanied by an enhanced expression co-stimulatory receptors in CD8 T cells of PD patients, while in PR patients they displayed a more exhausted phenotype. Interestingly, upon treatment with nivolumab, PD patients also showed a drop in the frequency of CD4 T cells expressing PD1 and BTLA, which may represent antigen-experienced helper T cell. Our study demonstrates that numbers of CD8 T cells as well as frequencies of CD8 and CD4 T cells with defined co-signaling signatures in peripheral blood are associated with response to nivolumab in NSCLC patients.

WS.A3.03.02
Circulating CD4 senescent T cells stratify clinical responses to PD-L1/PD-1 immune checkpoint inhibitors in NSCLC
M. Zuzul1, H. Aroasan1, M. Gato2, G. Fernández44, R. Vera2, G. Kochan2, D. Escors44;
1Navarroamibodi, Pamplona, Spain; 2Compleno Hospitalario de Navarra, Pamplona, Spain; 4University College London, London, United Kingdom;
Background: PD-L1/PD-1 blockade immunotherapy are demonstrating promising clinical outcomes in different neoplasms, although response rates are low and no accurate biomarkers of response have been discovered. Senescent T cells (Tsen) accumulate with age and comprise a large pool of antigen-specific T cells. Here, the impact in terms of survival (in months) and clinic-pathological variables of circulating CD4 T cells with defined senescent phenotype was assessed. Methods: A prospective small-scale study was conducted in 33 non-small cell lung cancer (NSCLC) patients treated with PD-L1/PD-1 immune checkpoint inhibitors. Baseline Tsen and their dynamic changes during treatment were quantified from peripheral blood samples and correlated with clinical efficacy based on RECIST criteria. Results: In our cohort study, patients with Tsen baseline values below 40% (negative baseline profile accounting to 52% of patients) had an ORR of 0% and 6 weeks PFS, in contrast to the remaining patients with a 37.5% response rate. Two main dynamic patterns of responses were identified. Increase in Tsen after the first cycle of therapy was always associated to progression (pattern 1, Tsen "burst"), while decrease (pattern 2) significantly correlated with responders. Hyperprogression was found in patients with a negative baseline profile and highly significant systemic CD4 Tsen bursts. Conclusions: Quantification of CD4 Tsen from routine blood samples provides an accurate predictive biomarker of responses with highly significant stratification power in NSCLC. Patients with a negative Tsen baseline profile did not respond to PD-L1/PD-1 immune checkpoint inhibitors or exhibited hyperprogressive disease. Enrolment of these patients for PD-L1/PD-1 blockade monotherapy should be avoided if other therapies are available.

WS.A3.03.03
CD103 tumor infiltrating lymphocytes as a prognostic factor in colon cancer patients
s. M. Talhouni1, J. Ramage1;
Nottingham City Hospital, Nottingham, United Kingdom;
Background: CD103 (β7 integrin) is expressed on human gastrointestinal and skin-homing CD8+ T cells, which are currently being explored as potential vaccine candidates.
CD103 TILs are present in both colon and rectal cancer patients. However, Intraepithelial CD103 was associated with better overall survival in right colon cancer patients (p=0.01) in comparison to left sided colon and rectal cancer patients (p=0.665 and 0.818, respectively). Conclusion: We hypothesise that increased mutational rates in the right sided colon tumours improved the prognostic role of CD103+ antigen presenting TRM cells in comparison to the left colon/rectal tumours.

WS.A3.03.04
Foligrastim enhances T-cell clearance by anti-thymocyte globulin exposure after unrelated cord blood transplantation
C. de Konig1, J. A. Gabelich2, J. Langenhorst3, R. Admiral4, J. Kuball3, J. J. Boelens4, S. Nierkens5
1University Medical Centre Utrecht, Utrecht, Netherlands; 2Wilhelmina Children's Hospital, Utrecht, Netherlands;
Residual anti-thymocyte globulin (ATG, Thymoglobulin) exposure after allogeneic hematopoietic (stem) cell transplantation (HCT) delays CD4+ T-cell immune reconstitution [CD4+ IR], subsequently increasing morbidity and mortality. This effect seems particularly present after cord blood transplantation (CBT) compared bone marrow transplantation (BMT). Tummelwes University, Budapest, Hungary. We investigated the effect of active-ATG exposure on CD4+ IR by BMT in 275 patients (CBT, n=155, BMT, n=120; median age 7.8 years; range 0.16-19.2) receiving their first allogeneic HCT between Jan-2008 and Sept-2016. Multivariate log-rank tests (with correction for covariates) revealed that CD4+ IR was faster after BMT than with CBT (<0.001), but not after BMT (p=0.74). To decipher this, we performed ATG-titration and -cytotoxicity experiments using CB- and BM-graft derived T-cell subsets, B, NK cells and dendritic and T-cell-depleted mononuclear cells. No differences were seen. Our cohort was Foligrastim treatment (only given after CBT). We found that Foligrastim (G-CSF) exposure highly increased neutrophil-mediated ATG-cytotoxicity, by 40-fold (0.5 vs. 20%, p<0.002), which explained the enhanced T-cell clearance after CBT. These findings imply revision of the use (and/or timing) of G-CSF in patients with residual ATG exposure.

workshops
WS.A3.03.05

Next-generation antigen receptor sequencing of paired diagnosis and relapse samples of B-cell acute lymphoblastic leukemia: an algorithm for Minimal Residual Disease target selection

P. Thrunseif1, M. de Bièr2, D. van Zessen3, V. de Hoas2, A. R. Stubbs2, V. H. J. van der Velden2

1Department of Immunology, Erasmus MC, Rotterdam, Netherlands, 2OCGG, The Hague, Netherlands, 3Department of Bioinformatics, Erasmus MC, Rotterdam, Netherlands.

Antigen receptor gene rearrangements are frequently applied as molecular targets for detection of minimal residual disease (MRD) in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) patients. Since such targets may however be lost at relapse, appropriate selection of antigen receptor genes as MRD-PCR target is critical. Recently, next-generation sequencing (NGS) - much more sensitive and quantitative than classical PCR-heteroduplex approaches - has been introduced for identification of MRD-PCR targets. In this study, we evaluated 42 paired diagnosis-relapse samples by NGS (IGH, IGK, TRG, TRD, TRB) to design an algorithm for selection of antigen receptor gene rearrangements which are most likely to remain stable at relapse. Overall, only 393 out of 1446 (27%) clonal rearrangements were stabled between diagnosis and relapse. If only index clones with a frequency >5% at diagnosis were taken into account, this number increased to 65%, including only index clones with an absolute read count >10'000, indicating truly major clones, further increased the stability to 84%. Over 90% of index clones at relapse were also present as microclone at diagnosis. Together, our data provide detailed information about the stability of antigen receptor gene rearrangements, on which we propose an algorithm for selecting stable PCR targets for MRD monitoring, which should enable successful detection of relapse in >95% of BCP-ALL patients.

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WS.A3.03.06

Plasma-derived exosomes in head and neck squamous cell carcinoma patients as potential biomarkers of response to immune therapies

M. Thoerardt1, T. Hoffmann2, R. Ferris2, T. Whitehead2

1Department of Otolaryngology-Head and Neck Surgery, University of Ulm, Germany, Ulm, Germany, 2Department of Otolaryngology-Head and Neck Surgery, University of Pittsburgh, Pittsburgh, PA, USA, Pittsburgh, United States, 3Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, Pittsburgh, United States.

Background: Circulating exosomes play a key role in immune suppression and disease progression. To evaluate their role as biomarkers of response to immunotherapy, we monitored changes in the cargo of exosomes from plasma of patients with head and neck squamous cell carcinoma (HNSCC) treated with Cetuximab, Ipiplimumab and IMRT.

Methods: Patients (n=18) with advanced HNSCC enrolled in the phase I clinical trial (NCT01935921) donated plasma at baseline and during (week 5, 14) immunotherapy. Exosomes were isolated by size exclusion chromatography and were separated into T-cell derived and non-T-cell derived exosomes by immunoaffinity capture. On-bead flow cytometry was used for detection of CTLA-4, PD-1 and CD15s (Treg marker) on exosomes. To immunocapture tumor-derived exosomes (TX), a microarray containing 4 antibodies specific for antigens overexpressed on HNSCC was used. Results were correlated to patients’ outcome. Results: All patients had high TX levels at baseline with a decrease at week 5. However, in patients who recurred (n=5), TX levels increased at week 14; in contrast, TX levels in 13 patients responding to therapy remained low. PD-1 levels in CD1(+) exosomes (exosomes derived from CD8+ T-cells) were elevated at week 5 in patients with recurrence but significantly decreased at week 5 in non-responders. However, a drastic decrease of CTLA4 occurred during therapy. Levels of CD15s were elevated at week 5 only in non-responders.

Conclusions: Exosomes in plasma of cancer patients treated with immune therapies may serve as biomarkers of early response to treatment.

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WS.A5.01

Germlinal center reactions

WS.A5.01.01

Follicular dendritic cells originate from subepithelial mesenchymal cells in Peyers’ patches

A. Pradat1, V. Kaliora2, G. Koliatis2

1BSRC Alexander Fleming, Vari, Greece, 2National and Kapodistrian University of Athens, Athens, Greece.

Peyer’s patches (PPs) are lymphoid organs that are located in the small intestine and play an important role in gut immunity. B and T lymphocytes are the main cell populations, which are segregated into two different lymphoid areas, driven by the presence of two major mesenchymal populations: fibroblastic reticular cells in the T cell area and follicular dendritic cells (FDCs) in the B cell area. Our group has previously shown that Collagen VI (ColVI)-Cre mice are a useful new tool for PP FDC analysis. Here, we used multicolor fate mapping to address whether follicular dendritic cells are derived from epithelial progenitors. Using a combination of confocal and light sheet fluorescence microscopy, we dissected the ontogeny and dynamics of this cell population. Analysis of adult PP from ColVI-Cre mice revealed the presence of monocloned cell columns connecting subepithelial mesenchymal cells and FDC networks, pointing towards an ontogenetic relation between them. To discern the directionality of this relation, we studied PP organogenesis. At embryonic day 18.5, ColVI-Cre+ cells appeared as a single cell layer underneath the epithelium. During the first week of life, these cells proliferated, migrated into the muscle layer and differentiated into FDCs. Interestingly, this migration/differentiation process was lymphoid receptor (LtβR)+/Tnf receptor 1 (TnfR1) dependent, since deletion of LtβR in ColVI-Cre+ cells in the LtβR+/- adult mice restricted to a single cell layer under the epithelium. In contrast, specific deletion of Tnf receptor 1 (TnfR1) in ColVI-Cre+ cells allowed their migration but blocked their differentiation into FDCs. To summarize, we demonstrated that during PP development, FDCs arise from subepithelial mesenchymal cells in a LtβR/TnfR1 dependent manner.

WS.A5.01.02

Novel branched proximity hybridization assay to quantify nanoscale protein-protein interaction

S. Zheng1, M. Mitteer2, M. Reth3, M. Cavallari3, J. Yang4

1Institute of Biology III, Freiburg, Germany, 2Max-Planck-Institute of Immunobiology and Epigenetics, Freiburg, Germany.

Recent studies suggest that membrane proteins are pre-clustered and highly organized in nanometer (nm) distances. This cell surface nanoscale protein organization plays an essential role in receptor signaling and signal propagation in both healthy and neoplastic cells. Due the current activation limitation, it is still difficult to study this nanoscale protein organization. To tackle this challenge, we have developed a new branched proximity hybridization assay (bPHA). In this assay, target proteins are first bound by specific antibodies, and quantitative manner. Deploying bPHA, we reliably detected the intermixing of B cell receptor isotypes upon B cell stimulation. We were also able to measure the intracellular antibody fragments (Fab, scFv, VHH) or aptamers coupled to specially designed (plus and minus) oligonucleotides. A nanoscale distance between two target proteins places the antibody fragments in close proximity, allowing them to activate Syk kinases. We validated this approach using a nanoscale distance between BCR isotypes and Syk. Using this approach, we could quantify the nanoscale proximal interactions between BCR isotypes and Syk in a quantitative and quantitative manner. Deploying bPHA, we reliably detected the intermixing of B cell receptor isotypes upon B cell stimulation. We were also able to measure the intracellular dynamics of Syk kinase recruitment to the BCR signaling subunit after treatment of B cells with different stimuli.

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WS.A5.01.03

Reconstitution of T-cell synaptic ectosomes unveils vesicular CD40L density as a critical determinant in the feed forward activation of antigen presenting cells

P. F. Cifuentes-Donaoro1, D. G. Saliba1, S. Volv1, S. Balint1, K. Karschewskaya1, E. Compeere1, M. Tagnoni1, E. O’Neill1, M. L. Dustin2

1The Kennedy Institute of Rheumatology, University of Oxford, Oxford, Oxford, United Kingdom, 2Department of Oncology, University of Oxford, Oxford, United Kingdom.

Extracellular vesicles are important intercellular communication elements across tissues. Recently, we have described synaptic ectosomes (SE) as a specialized type of extracellular vesicle that both form in response to antigen receptor stimulation and are released into the synaptic cleft by T-cells. However, the nature and functionality of the protein cargo of SE and their role in T-cell activation and signaling are still unknown. To study this nanoscale protein organization, we have developed a novel branched proximity hybridization assay (bPHA). In this assay, target proteins are first bound by specific antibodies, and antibody fragments (Fab, scFv, VHH) or aptamers coupled to specially designed (plus and minus) oligonucleotides. A nanoscale distance between two target proteins places the antibody fragments in close proximity, allowing them to activate Syk kinases. We validated this approach using a nanoscale distance between BCR isotypes and Syk. Using this approach, we could quantify the nanoscale proximal interactions between BCR isotypes and Syk in a quantitative and quantitative manner. Deploying bPHA, we reliably detected the intermixing of B cell receptor isotypes upon B cell stimulation. We were also able to measure the intracellular dynamics of Syk kinase recruitment to the BCR signaling subunit after treatment of B cells with different stimuli.

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WS.A5.01.04

In follicular regulatory T cells Nfatc1 is essential for homing to germlinal centers


1Institute of Pathology, Julius-Maximilians-University of Wuerzburg, 97080 Würzburg, Germany, 2Department of Tumor Genetics and Immunogenetics, Max-Delbruck-Center for Molecular Medicine (MDC), 13092 Berlin, Germany, 3Institute for Immunology, University Medical Center, University of Münster, 45122 Münster, Germany, 4Institute for Virology and Immunology, University of Würzburg, 97074, Germany.

Plasma cells, secreting class-switched antibodies with high affinity, are the product of the germinal center reaction (GCR). The GCR is supported by CD4+CXCR5+ follicular T-helper cells (Tfh), and controlled by CD4+CXCR5−/FOXP3+ follicular regulatory T-cells (Tr). Expression profiling revealed high expression of the “Nuclear Factor of Activated Cells 1” (Nfatc1) in follicular T cells, confining Tr as well as Tfh. The alation of Nfatc1 in all T cells, but also in Foxp3-expressing cells only, led to an increase in the GCR. This effect was due to an impaired homing of the Tfh population to the B-cell follicle, because Tfh cells specifically failed to upregulate the homing receptor CXCR5.
In T<sub>cm</sub> cells - in contrast to T<sub>eff</sub> cells - “lymphocyte-produced maturation protein” (Blimp-1) is highly expressed in line with being a hallmark of effector regulatory T-cells. Our data indicate that Blimp-1 directly represses the expression of Crex5 in T<sub>eff</sub>. However, it supports the recruitment of Nfatc1 to Crex5 by protein-protein interaction, by those means cooperating with Nfatc1 for transactivation of Crex5. In line, overexpressing constitutive active Nfatc1 in post-thymic T cells supported Ccx5 expression on Tregs. Interestingly, the numbers in T<sub>cm</sub> and germinal center B cells were so deeply reduced that a surplus in Nfatc1 might even enhance the effector function of T<sub>cm</sub> cells. In sum, Nfatc1 is essential for overcoming Blimp-1-mediated repression of Crex5 and therefore for holding of T<sub>cm</sub> cells, which control the GCR, an essential part of the humoral immune response.

**WS.A4.01.05**

Ectopic lung resident germinal centres are formed during chronic house dust mite driven allergic airway disease in a T follicular helper cell dependent manner

Imperial College London, London, United Kingdom.

**Introduction:** Allergic asthma is a disease of chronic allergen exposure, characterised by airway inflammation, airway hyperresponsiveness and allergen specific IgE. Germinal centres (GCs) are anatomically distinct structures, located primarily in secondary lymphoid organs, critical for antibody generation. Lymphocyte aggregates exist in the asthmatic lung, but their formation and contribution to disease is not understood. This study aims to understand ectopic GCs during chronic allergic airway disease (AAD).

**Method:** To establish chronic AAD, mice were repeatedly exposed to intranasal house dust mite for up to 5 weeks.

**Results:** GCs and T follicular helper cells (Tfh) were found in the mediastinal lymph nodes (mLN) and the lungs after 3 weeks of allergen exposure. Large B cell aggregates containing GC B cells, T cells and follicular dendritic cell (FDC) networks were identified in the lungs by confocal microscopy, indicative of an active GC. Sorting mLN and lung resident B cells revealed transcripts indicative of IgG1 and IgE switch in both compartments by qPCR, while IgA transcripts were only identified in the lungs, suggesting the lungs to also provide protective antibodies. Ectopic GCs were absent in Tfh deficient mice (Cd4<sup>−/−</sup>Bcl6<sup>−/−</sup>), which additionally lacked allergen specific IgE. Despite this, Cd4<sup>−/−</sup>Bcl6<sup>−/−</sup> mice had exaggerated Th2 cell biased AAD. Mixed bone marrow chimeras revealed a regulatory role for Tfh during AAD, rather than an intrinsic role for B cells in regulating Th2 cell differentiation.

**Conclusions:** Lung resident GCs form during chronic AAD in a Tfh dependent manner and Tfh are important regulators of disease.

**WS.A4.01.06**

Antigen stimulation of lymphoid cells supports alternative pathway of T Follicular helper cell differentiation by polarizing CD45RA<sup>+</sup> CD4 T cells into Tfh cells

R. Jeger-Madlot1, M. Pereira2, C. Richetta2, V. Quinouivi, P. Buffet2, D. Klatzmann2, A. Moris2, S. Graff-Dubois3;

Lymphocyte aggregates and antigen presentation systems to generate Tfh cell differentiation promotes B cell maturation. In chronic infections, Tfh cell frequency is increased. Chronic antigen stimulation might promote Tfh cell differentiation leading to pathogenic antibodies. Understanding the pathways of Tfh differentiation and GC reaction under pathologic conditions is of particular interest to develop new therapeutic approaches. Tfh cells are not easily accessible, limiting their study in humans. Here, we provide a culture system to generate fully differentiated Tfh cells, allowing to understanding the Tfh biology. Our culture system use human splenocytes stimulated with a superantigen and suitable cytokines.

We showed that induced Tfh cells present hallmarks of autologous Tfh cells, expressing Tfh markers and promoting B cell maturation. Compared with PBMC, Tfh cell differentiation is much more efficient with splenocytes in terms of quantity. This suggests that lymphoid environment promotes Tfh differentiation. Using this culture system, we demonstrated that Tfh could differentiate from CD45RA<sup>+</sup> CD4 T cells and naive T cells. We are currently performing experiments to characterize the functional properties of Tfh cells generated from naive and repolarized CD4<sup>+</sup> T cells. To our knowledge, this is the first evidence of a CD4 T cell repolarization into Tfh cells in humans. CD<sup>4</sup> T cell repolarization into Tfh cells might contribute to immune response, Tfh cell inflammatory diseases where lymphoid structures are exquisitely developed. In this culture system will provide insights on Tfh cell differentiation/functions. Looking ahead, this culture system will constitute an platform to test new therapeutic approaches.

**WS.A4.02 Regulation of B cell development and differentiation**

**WS.A4.02.01**

B cell positive selection is developmentally regulated during ontogeny by the heterochronic protein Lin28

V. Sanhee, S. Datta, T. Kristiansen, H. Åkerstrand, S. Soneji, E. Jaensson Gyllenbäck, J. Yuan;
Department of Molecular Medicine, Biocenter, University of Lund, Lund, Sweden.

While all T cell maturation requires self-antigen driven positive selection, most self-reactive B cells are subject to tolerance induction mechanisms. An exception to the rule is the CD<sup>+</sup> B-1 cell population known to be primarily of fetal and neonatal origin, generated and maintained on the basis of their self-reactivity. However, the mechanisms underlying their positive selection during early life and their subsequent developmental attenuation remains unclear. Here, we link surface CD5 levels to BCR self-reactivity within the B-1 compartment and show that CD5 is induced at the immature B cell stage during neonatal but not adult B cell maturation coinciding with B cell positive selection. Importantly, developmental induction of CD5 relies in a dose dependent manner on the heterochronic RNA-binding protein Lin28. Ectopic Lin28 reductively reinstitutes positive selection during adult B cell maturation and increases the progeny:precursor ratio of B cell selection by two fold as shown by cellular barcoding. Finally, our results uncouple the process of B cell positive selection from the semi-invariant repertoire and phosphatidylcholine reactivity characteristic for B-1 cells. Together, our data support a model in which developmentally restricted Lin28 expression potentiates a transient wave of B cell positive selection and thereby contributes to lifelong heterogeneity within the B cell pool.

**WS.A4.02.02**

The BHLH transcription factor TCF<sub>L</sub> and its sofar CHA differently modulate c-MYC-dependent B cell proliferation

I. Sánchez-Gómez, J. Galán-Martínez, M. Maza, K. Stamatakis, N. Girón, M. Fresno;
Centro de Biología Molecular Severo Ochoa, Madrid, Spain.

Modern lifestyle and increased average lifespan are causing a world-wide increase in cancer rates. c-MYC basic Helix-Loop-Helix (bHLH) transcription factor is key in the genesis of several types of cancer. Deregression of c-MYC has been frequently associated with aggressive lymphomas and adverse clinical outcome in B-cell malignancies. Transcription factor TCF<sub>L</sub> and its isoform CHA are also members of BHLH family. Since c-MYC and TCF<sub>L</sub> belong to the BHLH family they may be able to form heterodimers, thus the aim of this study is to analyze whether TCF<sub>L</sub>/CHA interact with c-MYC and if it also regulates its functionality in a cancer environment. Our results showed a direct interaction of c-MYC and CHA by co-immunoprecipitation assays. Then, we performed gene expression studies to analyze the effect that the overexpression of CHA in presence or absence of c-MYC and found inhibition c-MYC target genes expression by CHA. In addition, TCF<sub>L</sub>/CHA stably silenced cell lines were generated that confirmed our hypothesis about the modulatory role of TCF<sub>L</sub> and CHA in c-MYC mediated responses. Finally, protein expression analysis in acute lymphoblastic leukemia-like activated B cells showed differential effects of TCF<sub>L</sub> and CHA in cell proliferation. In summary, we found that CHA downregulates c-MYC activity and as a result it reduced cell tumorigenocity. On the contrary, TCF<sub>L</sub> expression was induced during B cell activation and was related to an immature stage contributing to a tumorigenic phenotype. Further studies may unravel CHA as a possible therapeutic target not only in cancer but also in leukemia.

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PI3Kδ hyper-activation in B cells promote increased susceptibility to S. pneumoniae airway infection through an antibody independent mechanism

A. Stark1, A. Chandrar1, R. Alam2, K. Okkenhaug2;
1Division of Immunology, Department of Pathology, University of Cambridge, CB2 1QQ, Cambridge, United Kingdom, 2Babraham Institute, Cambridge, CB22 3AT, UK, Cambridge, United Kingdom.

The PI3K<sub>δ</sub> signalling pathway is critical for normal immune cell development and function. Gain of function mutations affecting the p110<sub><i>δ</i></sub> catalytic- or p85<sub><i>α</i></sub> regulatory subunits causes Activated PI3K<sub>δ</sub> delta Syndrome (APDS), a primary immunodeficiency characterised by severe recurrent respiratory infections often caused by <i>S. pneumoniae</i>. We generated conditional knockout mouse models of PI3Kδ hyper-activation (PI3K<sub>δ</sub><sup>hyfactor</sup>) and PI3Kδ inactivation (PI3K<sub>δ</sub><sup>inact</sup>) to study the role of PI3Kδ signalling in the immune response to respiratory infection. Germine and B cell, but not T cell or myeloid, restricted PI3Kδ hyper-activation increases susceptibility to <i>S. pneumoniae</i> lung infection in PI3K<sub>δ</sub><sup>hyfactor</sup> mice. Kinase-inactive PI3K<sub>δ</sub><sup>inact</sup> mice were not more susceptible to infection, despite lacking natural antibody against <i>S. pneumoniae</i>. Furthermore, PI3Kδ hyper-activation does not limit natural antibody levels or an antibody response to Pneumovax, a 1-independent vaccine. Mice lacking mature B cells (μMT) are also protected against acute disease but fail to clear the infection, highlighting the pathological role of B cells in this model.

These data indicate that, while antibodies are important in the immune response to <i>S. pneumoniae</i>, B cells can play an antibody independent detrimental role during acute lung infection, and this effect is exacerbated by PI3Kδ hyper-activation. Indeed, we found an atypical II-10 producing CD19<sup>B220</sup> B2 cell subset which is significantly expanded in PI3K<sub>δ</sub><sup>hyfactor</sup> mice and absent in PI3K<sub>δ</sub><sup>inact</sup> mice. Ongoing work focuses on elucidating the mechanism whereby these cells can contribute to pathology in the early phase of <i>S. pneumoniae</i> infection.

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FUNCTIONS OF ZBTB24 IN B CELLS AND THE GENERATION OF Tfh CELLS

ZBTB24 has recently been identified as the causative gene in patients with Immunodeficiency, Centromeric Instability and Facial Anomalies syndrome type 2 (ICF2), a rare autosomal recessive disease. Most ICF2 patients harbor ZBTB24 nonsense mutations, and suffer from recurrent respiratory and gastrointestinal infections due to hypogammaglobulinemia, most likely due to the lack of germinal center (GC) structure and circulating CD19CD27 memory B cells (Bmem). ZBTB24 belongs to the large ZBTB family of transcription factors that harbor CUB-like (complement C1r/C1s, complement 2, and Ig) domains at the amino terminus and DNA-binding zinc-finger motifs at the carboxy terminus. It has been shown that ZBTB24 is highly expressed in human B-cell compartment. Knockdown of endogenous ZBTB24 hampers the cell-cycle progression in human GC-derived B lymphoma Raji cells via upregulating the expressions of IRF4 & PRDM1, two essential transcriptional factors involved in GC-reactions. Moreover, ZBTB24 exerts these functions independent of BCL6 as it neither heterodimerizes with nor antagonizes BCL6's repression/activation of BCL6L. Collectively, ZBTB24 appears to control the in vivo Bmem development via regulating the proliferation and/or terminal differentiation of human B cells. Intriguingly, despite the early embryonic lethality in conventional zbtb24-deficient mice, conditional-knockout of zbtb24 in murine B cells has no significant impact on in vivo antibody responses upon immunization with T-cell-dependent antigen. Thus, functions of ZBTB24 in B cells seem to differ in mice and humans.

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STEM CELL BASED GENE THERAPY FOR RECOMBINASE DEFICIENT-SCID

In this study we investigated the compartment-specific sphingolipid modulation for the development of colitis-associated colorectal cancer (CAC) and found that sphingolipids can account for both mechanisms: inflammation-induced cancer and cancer-induced inflammation. In vitro sphingolipid knockdown of intestinal and colonic epithelial cells provoked immediate occurrence of epithelial-driven tumors. Carcinogenesis was accompanied by an IL-12/IL-23 shift and sphingosine kinase 1, S1P receptor 2 and epidermal growth factor receptor upregulation with a consecutive development of a T h2-driven microenvironment. Moreover, both knockdown models showed distinct regulation of lymphopenia and neutrophilia, different from the global Sgpl1 knockdown. Conclusion: Our results demonstrate that cell-type-specific sphingolipid modulation contributes to the development of either inflammation-induced cancer or cancer-induced inflammation.
Blood transcriptomic analysis shows how recall innate responses are modulated by the use of an adjuvant at priming

E. Pettini1, F. Santoro2, D. Kazmir1, A. Ciabattini1, F. Forino1, G. Giffanti1, I. Eversroed, P. Andersen3, G. Pazzi1, D. Medaglini1;
1Laboratorio di Microbiologia Molecolare e Biotecnologia (LA.M.M.B.), Siena, Italy, 2Emergency Medicine Center, Emory University, Atlanta, GA, United States, 3Department of Medicine, Oslo University Hospital and University of Oslo, Oslo, Norway

Transcriptomic profiling of the immune response induced by vaccine adjuvants is of critical importance for the rational design of vaccination strategies. In the present study, we investigated how the vaccine adjuvant used for priming modifies the way the immune system responds to the re-exposure to the vaccine antigen alone. mRNA sequencing was performed on blood samples collected after priming and boosting from mice primed with the vaccine antigen H56 of Mycobacterium tuberculosis administered alone or with the CAF01 adjuvant and boosted with the antigen alone. Gene expression analysis 2 days after priming showed that the CAF01 adjuvanted vaccine induced a stronger upregulation of the innate immunity modules compared to the unadjuvanted formulation. The immunostimulant effect of CAF01 adjuvant, used for priming, was clearly seen also one day after boosting, with activation of blood transcriptomic modules related to innate immune response, such as monocyte and neutrophil recruitment, activation of antigen presenting cells and interferon response. The analysis of the immune response showed a higher frequency of HS6-specific CD4+ T cells and germinal center B cells in draining lymph nodes and a strong HS6-specific humoral response in mice primed with H56 + CAF01. Transcriptomic analysis of isolated HS6-specific CD4+ T cells was also conducted to profile gene expression in the mature antigen-specific helper T cell population upon vaccination. These data indicate that the adjuvant used for priming strongly re-programs the innate immune response that, upon boosting, results in a stronger recall of the innate response essential for shaping the downstream adaptive response.

WS.AS.01.04
DNGR-1 dampens neutrophil recruitment to damaged tissues, fostering disease tolerance upon infection

C. DEL FRESNO SANCHEZ1, P. Szaz-Leal1, M. Enamorado1, S. Wculek1, S. Martinez-Cano1, N. Blanco-Menendez1, O. Schulze1, M. Gallizioli1, F. Mird1, E. Cano1, A. Planas1, C. Reis e Sousa1, D. Gajdasik1;
1FUNDACION CNIC, MADRID, Spain, 2The Francis Crick Institute, London, United Kingdom, 3Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, 4Unidad Funcional De Investigacion De Enfermedades Crónicas, Instituto De Salud Carlos III, Majadahonda, MADRID, Spain

Introduction: DNGR-1 (Cleckl) is a dead-cell receptor mainly expressed on type-I dendritic cells (DC1s) implicated in cross-presentation of dead cell-associated antigens to CD8+ T cells. We propose that DNGR-1 also impacts on innate immune responses.

Methods: WT mice and DNGR-1-/- were subjected to sterile (acute necrotizing pancreatitis) and infectious (systemic Candida albicans infection) tissue damage models, analyzing myeloid infiltration and the tissue injury extent. Same models were addressed in anti-DNGR-1-treated WT mice or DNGR-1-deficient mice lacking adaptive immunity. To address the role of neutrophils to the damaged organs, mice were either WT or DNGR-1-deficient with neutrophil-depleting antibodies. The inflammatory response of DC1s to some PAMPS was analyzed after in vitro exposition to a ligand for DNGR-1. The implication of SHP-1 was addressed in vitro through a chemical inhibitor. The expression of the neutrophil chemoattractant Mip-2/CXCL2 was analyzed in sorted haematopoietic populations from C. albicans-infected kidneys.

Results: DNGR-1 absence or blockade led to exacerbated caerulein-induced pancreatitis. Similarly, DNGR-1-deficient settings increased pathology during Candida infection without affecting fungal burden, suggestive of a disease tolerance-related process. Both exacerbated responses were independent of adaptive immunity and attributable to increased neutrophilia. Ligand engagement by DNGR-1 activates SHP-1 to dampen inflammatory responses, decreasing the expression of the neutrophil chemokine MIP-2/CXCL2. Among the renal immune infiltrate of Candida-infected DNGR-1-deficient mice, DC1s were the only population overexpressing Mip-2/CXCL2.

Conclusions: Tissue damage sensing by DNGR-1 in DC1s negatively regulates Mip-2/CXCL2 expression, reducing host-damaging neutrophil infiltration. Upon infectious conditions, this immunomodulation occurs without affecting pathogen burden, suggesting that DNGR-1 could promote disease tolerance.

WS.AS.01.05
Toll-like receptor signalling induces a temporal switch from inflammatory towards a more resolving lipid profile in monocyte-derived macrophages

J. von Hegedu1, M. Nejink1, T. Huizinga1, M. Kloppenburg1,2, M. Giera1, R. Toes1, A. Loos-Facinsky1;
1Department of Rheumatology, 2333 ZA, Netherlands, 2Center for Proteomics and Metabolomics, 2333 ZA, Netherlands

Background: Inflammation is a tightly regulated process that usually resolves spontaneously. Dysregulation of this process can lead to chronic inflammation. Several cells and soluble mediators, including lipid mediators, regulate the course of inflammation and its resolution. Previous data suggest a temporal lipid mediator switch from pro-inflammatory lipid mediators at the start of inflammation towards specialized pro-resolving lipid mediators (SPM) during the resolution phase of inflammation. It is, however, unclear which signals initiate secretion of SPM and the resolution process. Macrophages are key players in regulating tissue inflammation through secretion of soluble mediators , including lipid mediators.

We hypothesize that the initiation of resolution is orchestrated by macrophages in response to persistent inflammatory stimuli.

Methods: M1 polarized monocyte-derived macrophages were stimulated with LPS for different periods of time. Changes in lipid profile were measured using liquid chromatography coupled to tandem mass spectrometry. In parallel, expression of cyclooxygenase and lipoxigenases-15 was determined using qPCR. Additionally, IL-10 and TNFa ELISA’s were performed on monocyte-derived macrophages that were pre-incubated with lipid mediators.

Results: Twenty-four different lipids were detected in LPS-stimulated macrophages. Cyclooxygenase-derived pro-inflammatory prostaglandins were observed in the first six hours of stimulation. Interestingly, a switch towards the 15-lipoxygenase SPM precursors 15-HETE and 17-HDHA was observed after 24h. The mRNA expression of cyclooxygenase and lipoxigenase genes was analyzed. In parallel, macrophages were stimulated with LPS with or without 15-HETE or 17-HDHA for 24h. The expression of cyclooxygenase and lipoxigenase genes was analyzed.

Conclusions: Macrophages can initiate the resolution of inflammation in response to persistent inflammatory stimuli.
**WORKSHOPS**

**WS.A5.02.01**

Mature CD10+ and immature CD10− neutrophils display opposite effects on T cells


1Department of Medicine, Division of General Pathology, University of Verona, Verona, Italy, 2Department of Medicine, Division of Internal Medicine, University of Verona, Verona, Italy, 3Interdepartmental Laboratory of Medical Research, University of Verona, Verona, Italy, 4Interdepartmental Laboratory of Medical Research, Applied Research on Cancer-Network, University of Verona, Verona, Italy, 5Rheumatology Unit, Division of General Medicine, Sacro Cuore Hospital of Negrar, Verona, Italy, 6Rheumatology Unit, Division of General Medicine, Sacro Cuore Hospital of Negrar, Verona, Italy, 7Transfusion Medicine Department, Integrated University Hospital, Verona, Italy, 8Department of Surgery, Division of General Surgery “A,” University of Verona, Verona, Italy, 9Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.

The identification of neutrophil populations, as well as the characterization of their immunoregulatory properties, is an emerging topic under extensive investigation. In such regard, the presence of circulating CD66+ neutrophils, exerting either immunosuppressive or proinflammatory functions, has been described in several acute and chronic inflammatory conditions. However, due to the lack of specific markers, the precise phenotype and maturation status of these neutrophils remain unclear. Herein, we report that a CD10+ neutrophil marker that, within CD10+ neutrophils, presents strong expression of circulating CD66+ neutrophils has been recognized that, in inflammatory conditions, clearly distinguishes the mature from the immature ones. Accordingly, we observed that the previously described immunosuppressive neutrophil population that appears in the circulation of granulocyte colony-stimulating factor (G-CSF)-treated donors (GDs) consists of mature CD66+ CD10+ neutrophils displaying an activated phenotype. These neutrophils inhibit T cell functions via CD95L-CD95 interaction. In contrast, we found that immature CD66+ CD10− neutrophils, also present in GDs, display an immature morphology, promote T-cell survival and functionality. Altogether, our findings uncover that in GDs, circulating mature and immature neutrophils exert opposite immunoregulatory properties. Therefore, CD10 might be used as a phenotypic marker discriminating neutrophil populations present in patients with acute or chronic inflammatory conditions, as well as facilitating their isolation, to better define their specific immunoregulatory properties. (Blood. 2017;129(10):1343–1356)

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Tight translational control of glycolysis and fatty acid metabolism regulates the transition of CD4+ T cells from quiescence to metabolic remodelling

S. Ricciardi, N. Maffriani, R. Affreri, P. Calamita, M. Crasti, R. Muller, M. Paganini, S. A. Acremoglini, S. Biffi

1National Institute of Molecular Genetics, Milan, Italy, 2Helmholtz Institute for Pharmaceutical Research, Saarbrücken, Germany, 3Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy, 4Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy, 5Bioscience Department, Università degli Studi di Milano, Milan, Italy.

Upon antigen encounter via TCR, naïve cells undergo a metabolic reprogramming, which supports growth and imparts distinct functional fate, but the molecular basis for this is unclear. Integrating multiple “omics” analysis of human resting and naïve cells following activation, we discovered that T cells exert the transitional process through translational control. Here, we show that the premetabolic state of CD4+ T cells encoding for glycolytic and fatty acid synthesis factors, and present a unique metabolic profile. Upon TCR engagement, activation of the translational machinery leads to synthesis of GLUT1 protein that steers glucose entry. Next, translation of ACC1 mRNA, via elf4E, complements metabolic reprogramming toward an effector phenotype. Notably, inhibition of elf4E abrogates lymphocyte metabolic activation and differentiation, defining ACC1 as a key regulatory node. Our results demonstrate that translation is the mediator of T cell metabolic control and indicate translation factors as targets for novel immunotherapeutic approaches.

**WS.A5.02.03**

Regulatory and conventional T cell transcription factors are reciprocally controlled during Salmonella infection

S. Clay, A. Bravo Blas, D. Wall, M. MacLeod, S. Milling, Institute of Infection, Immunity and Inflammation, Glasgow, United Kingdom.

Peripherally induced FoxP3+ Tregs (pTregs) play an important role in controlling inflammation and maintaining homeostasis at mucosal sites. pTregs differentiate from conventional T cells and can express transcriptional factors (TFs) including T-bet, GATA3 and RORγT, markers used to identify Th1 helper (Th1) subsets. T-bet+ Tregs can selectively suppress T-bet+ Th1 cells but it is unclear whether Tregs expressing other TFs selectively inhibit corresponding Th subsets. It is also unclear whether this selective regulation influences T cell polarization. To address these questions we use a Salmonella enterica serotype Typhimurium (STM) strain that allows characterisation of antigen specific and total T cells in lymphoid and mucosal sites. One week after oral infection, an increased proportion of Th17 cells are found in the colon, with a reduced proportion of Tbet+ Th1 cells and increase in T-bet+ Tregs. Two weeks post-infection this dynamic is switched to a Th1 bias, with a reduced proportion of RORγT+ Th17 cells and increase in RORγT+ Tregs. This reciprocity between conventional Th1 cells and Tregs expressing the same TFs occurs in the colon and caecum but not in draining lymph nodes. These findings are consistent with the hypothesis that pTregs shape T cell responses by selectively suppressing Th subsets at effector sites. To test this hypothesis we adoptively transfer Tregs expressing specific TFs into Treg-depleted recipients and assess the impact on the Th cell response. This will reveal whether pTregs are capable of subset-specific regulation of Th cells, highlighting their potential utility for targeted therapeutic approaches.

**WS.A5.02.04**

CD28 costimulation and not TCR controls the effector functions of activated CD4 T cells

B. Soskic, D. A. Glinos, D. M. Sansoni, G. Trynka

1National Institute of Molecular Genetics, Milan, Italy, 2Institute of Immunology and Transplantation, University College London, London, United Kingdom.

T cell response is initiated following interaction of antigen presenting cells. The T cell receptor (TCR) determines the specificity of the response and the CD28 costimulatory receptor helps to ensure that activation does not occur upon recognition of self-antigens. It is widely thought that CD28 is required for activation of naive and not memory T cells. Here, we used functional genomics assays to investigate the role of costimulation via CD28 on gene expression programmes in human naive and memory T cells. We demonstrate that T-cell differentiation, cytokine and chemokine expression increase in response to CD28 in TCR intensity in both naive and memory cells. Strikingly, we observe that cell cycle and cell division are sensitive to CD28 in memory cells, but under TCR control in naive cells, in contrast to the paradigm that memory cells are CD28-independent. Using a combination of chromatin accessibility and enhancer profiling, we demonstrate that interferon response elements (IREs) and Blimp-1 motifs are enriched in naive and memory T cells in response to TCR. In contrast, memory cells initiate API1 transcriptional regulation only when both TCR and CD28 are engaged, implicating CD28 as an amplifier of transcriptional programmes in memory cells. Lastly, we show that CD28-sensitive genes are enriched in autoimmune disease loci, pointing towards the role of memory T cell activation through CD28 in autoimmune disease development. This study provides new insights into the impact of TCR and CD28 in the activation of human naive and memory CD4 cells.

**WS.A5.02.05**

The genome organizer Satb1 is required for the development of Th17 cells through regulation of Il-2 expression


1Molecular Immunology in Neurodegeneration, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany, 2Life & Medical Sciences institute (LIMES), University of Bonn, Bonn, Germany, 3PRECESE, Platform for Single Cell Genomics and Epigenomics, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany, 4Institute of Innate Immunity, University of Bonn, Bonn, Germany, 5Institute for Neuropathology, University of Münster, Münster, Germany, 6Department of Psychiatry and Psychotherapy, University of Münster, Münster, Germany, 7Institute for Pathology, Martin-Luther-University, Halle, Germany.

T cells play an important role in host defence and tissue homeostasis. T cell dysfunction is associated with multiple diseases such as increased susceptibility to pathogen infection, inflammatory and autoimmune diseases as well as cancer formation. Thus, it is critical to identify the common underlying mechanisms governing T cell differentiation and establish new approaches to influence their differentiation in disease settings. Here we describe the genome organizer special AF-rich-binding protein 1 (Satb1) as a critical regulator of Th17 cell development. We could show that Satb1 is highly expressed during Th17 cell differentiation and that a loss of Satb1 prevents Th17 cell development. Furthermore, expression of Satb1 in CD4+ T cells is required for the induction of autoimmune diseases, like experimental autoimmune encephalomyelitis (EAE) and inflammatory bowel disease. The formation of complex transcription factor networks, controlled through Satb1 and other transcription factors and chromatin modifications, is required for specific T cell function and lineage commitment. Using transcriptional and epigenetic characterization of Satb1-deficient CD4+ T cells, we could show that Satb1 mediates Th17 cell development by preventing Il-2 expression early during Th17 cell development. Satb1-dependent Il-2 regulation influences STAT proteins, the pioneer factors for T cell differentiation, thus affecting Th17 cell transcription factor network formation phenocopying Ahr ligation-mediated induction of Th17 cell differentiation. In line with this, activation of Ahr signaling in Satb1-deficient CD4+ T cells could rescue Th17 cell differentiation. Taken together, Satb1 is critical for the differentiation of T17 cells through epigenetic programming early during Th17 cell development. Thus, Satb1 may pose a novel therapeutic target for the treatment of T17 cell-driven autoimmune diseases.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 53
Unique metabolic requirements for Th1 helper 2 polarization by dendritic cells

L. Pergolizzi1, A. Sergiuschev1, A. J. van der Ham2, B. W. Minkel, M. Yazdanbakhsh3, M. N. Artyomov4, B. Everts5; 1Leiden University Medical Center, Leiden, Netherlands, 2ITMO University, Saint Petersburg, Russia Federation, 3Washington University School of Medicine, St. Louis, United States.

Dendritic cells (DCs) play a central role in the activation and polarization of T cell responses. We recently found that toll-like receptor signaling promotes a shift to glycolysis to support the anabolic demands of murine DC activation and effective priming of T cell responses. However, the metabolic requirements for polarization of distinct T helper cell (Th) responses by DCs, in particular Th2 responses, remain poorly defined. Based on unbiased global transcriptomic comparison of immature, Th1-, Th17- and Th2-priming human monocyte-derived DCs (hMDCs), we here report that suppression of genes involved in glycolysis is a key distinguishing feature of Th2-priming DCs. Consistent with these observations, in contrast to Th1- and Th17-priming DCs, Th2-priming DCs display low glycolytic rates and fail to increase glycolysis upon TLR stimulation. Importantly, blocking of glycolysis in DCs is sufficient to condition them for Th2 priming. Furthermore, based on unbiased analysis of integrated global metabolomic with transcriptomic data (ComBi-T), we additionally identified an activated UDP-GlcNAc module in Th2-priming DCs, which points towards an important role for N- and O-GlcNAcylation in Th2 priming by DCs. In line with these data, inhibition of O-linked GlcNAc transferase (OGT) impeded Th2 priming by DCs, without affecting their Th1- and Th17-priming capacity. Together, these findings suggest that DC-driven polarization of different T cell responses is dependent on the activation of distinct metabolic programs in DCs and highlights that metabolic manipulation of DCs could hold promise as a novel therapeutic approach to control immune-polarization in disease settings.

DCs and tissue-derived cellular responses

Engulfment and active shuttling of mast cell granules boosts dendritic cell functions

J. Kotrba1, J. Dudeck1, I. Frobel1, A. Dudeck2; 1Institute for Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany.

Mast cell granules (MCs) represent key effector cells of type I allergic reactions but also play an important role in host defense against pathogens. Despite increasing evidence for a critical impact of MCs on the induction of adaptive immunity, the underlying mechanisms are poorly understood. We therefore aimed at monitoring MC-DC interactions with neighboring dermal dendritic cells (DCs). We studied MC behavior and communication using intravital multiphoton microscopy of Mcp5-Cre reporter mice. Moreover, we targeted DCs for a strategy to stain secretory granules in vivo inside the intact MCs allowing for the detection of MC degranulation. To assess the activation of DCs, intravital imaging was combined with flow cytometry, sorting and ex vivo functional assays. Here, we demonstrate using intravital imaging, that dermal DCs engulf the intact dense core secretory granules exocytosed by MCs upon LPS-induced skin inflammation. Subsequently, the engulfed MC granules are actively shuttled to skin draining lymph nodes (LNs) and finally degraded inside DCs within the lymphoid tissue. Most importantly, DCs bearing MC granules show an advanced early migration to skin draining LN, a highly enhanced maturation and boosted T cell priming efficiency as compared to MC-granule-negative DCs. Consequently, we highlight a unique feature of peripheral MCs to impact on lymphoid tissue borne adaptive immunity over distance by modifying DC functionality via the delivery of granule-stored mediators.

Cross-dressing of mast cells with MHC II from dendritic cells during skin inflammation

J. Dudeck1, A. Medyukhina1, I. Frobel1, J. Kotrba1, M. Fitzgerald; 1Institute for Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany, 2Applied Systems Biology, Leibniz Institute for Natural Product Research and Food Quality, Hans-Knöll-Institute Jena, Jena, Germany.

Mast cells (MCs) and dendritic cells (DCs) are essential innate sentinels populating host-environment interfaces. Despite increasing evidence for a critical impact of MCs on DC functionality and the induction of adaptive immunity, the underlying mechanisms are poorly understood. Here, we studied the intercellular communication between MCs and dermal DCs during contact hypersensitivity (CHS) associated skin inflammation by means of longitudinal intravital multiphoton microscopy of MC/DC double reporter mice and extensive quantitative analysis. Further, the functional relevance of MC/DC interaction in vivo was assessed by flow cytometry analysis and functional readouts. We herein provide in vivo evidence that migratory DCs execute targeted cell-to-cell interactions with stationary MCs before leaving the inflamed skin to draining lymph nodes. During initial stages of skin inflammation, DCs dynamically scan MCs, whereas at a later stage, long-lasting interactions predominate. These innate-to-innate synapse-like contacts ultimately culminate in DC-to-MC molecule transfers including major histocompatibility complex II (MHC II) proteins enabling subsequent ex vivo priming of allogeneic T cells with a specific cytokine signature. The extent of MHC II transfer to MCs correlates with their T cell priming efficiency. Importantly, preventing the cross talk by preceding DC depletion decreases MC antigen presenting capacity and T cell driven inflammation. Consequently, we identify an innate intercellular communication arming resident MCs with key DC functions that might contribute to the acute defense potential during critical periods of migration-based DC absence.

CD86+ antigen-presenting B cells are increased in solid cancers and induce tumor antigen-specific T cell responses

K. Wenhof; 1, M. Thelen1, A. Lechner2, H. Schröfer; 1, M. van Bergwelt-Baildon; 1, 2, 3Center for Molecular Medicine Cologne, Cologne, Germany, 3General-, Visceral- and Tumor Surgery, University Hospital Cologne, Cologne, Germany, 4Department of Medicine III, University Hospital, LMU Munich, Munich, Germany, 5German Cancer Consortium (DKTK), Heidelberg, Germany, 6Comprehensive Cancer Center Munich (CCCIM), Munich, Germany.

B cell effector functions do not only include secretion of antibodies, but also presentation of antigen to T cells. Recently, a physiological B cell subset with strong immunostimulatory properties was described in humans. These antigen-presenting B cells (B*ap) are characterized by a high expression of CD86 and downregulation of CD21. B*ap are expanded following vaccination or under inflammatory conditions. We analyzed seven different tumor entities for the presence of B*ap by flow cytometry and found increased percentages in lung adenocarcinomas, head and neck squamous cell carcinomas, colorectal cancer, esophageal-gastric cancers and renal cell carcinomas. Confocal microscopy demonstrated that CD86+ B cells organize in tertiary lymphoid structures in the tumor microenvironment. Tumor antigen-specific B cells isolated from tumor-draining lymph nodes of cancer patients showed increased percentages of B*ap. Furthermore, we demonstrate a strong induction of tumor-specific T cell responses by B*ap using an antigen-specific fluorospot assay. Our results highlight the relevance of B*ap as professional antigen-presenting cells in cancer.

Beta2-integrins restrict dendritic cell migratory phenotype through MRTFA/SRF signaling and a Syk-dependent epigenetic mechanism

C. Guenther1, M. Fusciloi, M. Ilander1, M. Sakolova1, T. Savoini1, L. Ustila1, S. Yiao2, M. Moser2, S. W. Morris2, V. Cerulli3, S. Tjokkander3, M. Vartainen1, S. C. Fagerholm1; 1Department of Biosciences, University of Helsinki, Helsinki, Finland, 2Department of Pharmacology, University of Helsinki, Helsinki, Finland, 3Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland, 4Department of Molecular Medicine, Max Planck Institute of Biochemistry, Munich, Germany, 5Department of Hematology-Oncology, St. Jude Children’s Research Hospital, Memphis, United States.

Dendritic cells (DCs) are the classic antigen presenting cells of the immune system. DCs switch from an adhesive, phagocytic phenotype to a migratory phenotype in response to stimuli such as LPS but also through unknown stimuli, resulting in spontaneous migration to lymph nodes. We have found that beta2-integrins regulate the migratory phenotype of DCs and their ability to induce strong immune responses. We used a beta2-integrin knock-in mouse model, which lacks the beta2-integrin-endophilin-3 interaction (TTT/AAA-beta2) and thus has non-functioning beta2-integrins, to investigate how beta2-integrins restrict DC function. Interestingly, we found that beta2-integrin KO BMDMs displayed reduced adhesion and traction force generation but increased 3D migration speed in vitro. We show that RhoA activation and F-actin polymerization is abolished in beta2-integrin KO DCs, which leads to a failure of MRTFA-A transcription factor to localise to the cell nucleus to co-activate genes with SRF. The integrin/RhoA/MRTFA/SRF pathway regulates DC adhesion, traction force generation and expression of chemokine receptors necessary for DC migration, eg CCR7. Furthermore, KO DCs displayed increased Syk activation and a Syk-dependent global increase in histone methylation (H3K4me3, characteristic of active genes). Inhibiting Syk and histone demethylases in WT DCs induced faster 3D migration. Utilizing a B16OVA tumor model, we show that DCs expressing dysfunctional integrins induced increased tumor rejection in vivo. Thus, beta2-integrin-mediated adhesion to the extracellular environment restricts DC migration and DC-mediated tumor rejection in vivo through MRTFA/ SRF signalling and a Syk-dependent epigenetic mechanism.
Expanded T-cell clones are present in the synovium before the clinical onset of rheumatoid arthritis

G. Balloretti1, P. L. Klarenbeek2, M. E. Doorenspleet2, M. J. de Hair1, B. C. van Schaik2, R. E. Essedt1, M. G. van de Sande4, D. M. Gerlag2, A. H. van Kampen2, F. Baar1, P. Tak1, N. de Vries2;
1Academisch Medisch Centrum, Amsterdam, Netherlands, 2Clinical Unit GlaxoSmithKline, Cambridge, United Kingdom, 3Leiden University, Genome Diagnostics, Leiden, Netherlands, 4GlaxoSmithKline, Stevenage, United Kingdom.

Introduction: In healthy individuals with RA-specific autoantibodies the presence of expanded B-cell receptor (BCR) clones in peripheral blood (PB) accurately predicts who will develop arthritis in the short term. Following up on these observations, we investigated whether T-cell receptor beta (TCRB) repertoire characteristics in PB and synovial tissue (ST) in this phase might also predict imminent onset of arthritis. Methods: Next-Generation Sequencing of the TCRB repertoire was performed on 20 randomly selected individuals with elevated IgM-RF and/or ACPA levels. Ten individuals did not develop arthritis during at least 3 years of follow-up, and 10 individuals did. PB and ST samples were analysed during the at-risk phase and again after onset of arthritis. Results: In the at-risk phase the synovium is already characterized by expanded TCRB clones, both in at-risk individuals that will and will not develop arthritis later. These clones persist during onset of arthritis. A higher impact of dominant later TCRB clones in synovial tissue at baseline was associated with longer time to arthritis (p=0.02). Conclusion: Expanded T-cell clones are present in the synovium in the at-risk phase regardless of future development of RA. They are maintained after onset of clinical disease. Combined with literature data, these observations show that T cell clones are already expanded in ST very early in disease, and suggest an overall regulatory role. Further studies are needed to characterize these clones.

WS.A5.03.06
Systems metabolic profiling reveals synergistic control of naive T cell priming by autophagy and mTOR

N. Franco1,2, L. Papagno1, A. Caputo1, V. Appoy1;
1Department of Molecular Medicine, Padova, Italy, 2CMI Paris, Inserm U1135, UPMC, Hospital Pitié-Salpêtrière, Paris, France.

96 Normal 0 false false false FR JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-copy-parent:""; mso-ansi-language:FR; mso-fareast-language:FR; mso-bidi-language:FR;}

Introduction: The metabolic processes that regulate the fate of distinct T lymphocyte subpopulations remain poorly defined, especially in humans.

Materials and methods: We used a systems approach to characterize the basal and activation-induced energetic requirements of naive and phenotypically-defined subsets of memory CD4+ and CD8+ T cells. To test the importance of metabolic pathways on primary responses, we used an original model of in vitro priming of epitope-specific naive CDB T cells. Priming was performed in the presence of different drugs affecting metabolic pathways, and the expansion and phenotype of epitope-specific CD8+ T cells assessed. Results: Profound metabolic differences were apparent as a function of lineage and differentiation status, both at rest and in response to stimulation via the T cell receptor (TCR). Of particular note, resting naive CD4+ and CD8+ T cells were largely quiescent, but rapidly upregulated diverse metabolic pathways after ligation of surface-expressed TCRs. Moreover, autophagy and the mTOR-dependent glycolytic pathway were identified as critical mediators of antigen-driven priming in the naive CD8+ T cell pool, the efficiency of which was dependent on the presence of neutral lipids and low levels of fatty acids. Conclusions: These observations provided a metabolic roadmap of the T cell compartment in humans and revealed potentially selective targets for novel immunotherapies (1-EndFragment).

WS.A6.01.01
CLIP upregulation on B cells associates with multiple sclerosis onset and is governed by autoimmunity risk allele CLEC16A

L. Rijvers1, M. Melief2, M. Stephan1, J. van Langelaar1, M. van der Vuurst de Vries1, A. F. Wierenga-Wolf1, M. van Ham1, R. Q. Hintzen3, M. M. van Luijten1;
1Department of Neurology, M C Center Erasmus, M G. van de Sande, A. Ozen1,2,3, S. Sari2, B. Dalgic2, I. Gursel2, M. Gursel4, L. Starrs1, M. de Hair1,2,3, M. G. van de Sande4, D. M. Gerlag2, R. Q. Hintzen3;
1Department of Neuroimmunology, Simon Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands. 2Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands. 3Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands. 4Department of Neurology, M C Center Erasmus, Erasmus MC, Rotterdam, Netherlands.

C-type lectin CLEC16A is located next to CITLA at a susceptibility locus for many autoimmune diseases, including multiple sclerosis (MS). We previously reported that CLEC16A is upregulated in MS and promotes the biogenesis of HLA-II peptide-loading compartments (MIIC) in myeloid antigen-presenting cells. Since T-cell activation by B cells is an important process.

WS.A6.01.02
CDD5deficient patients respond differentially to TLR, Inflammasome and patient eosinopoiesis stimulation before and after Eculizumab therapy

G. G. Kayi1, M. Yildrim3, L. Evli1, N. Becboyoglu1, I. C. Ayangoga1, A. Ozger1, S. Sar1, B. Dalgic2, M. Gursel3, I. Gursel4;
1Bilkent University, Ankara, Turkey, 2Middle East Technical University, Ankara, Turkey, 3Marmara University, Istanbul, Turkey, 4Gazi University, Ankara, Turkey.

CDD5 is a membrane bound protein whose deficiency has been recently defined as hyperactivation of complement, angiopathiic thrombosis, and protein-losing enteropathy (CHAPLE syndrome). It inhibits classical and alternative complement pathway while it acts and mediates activity of leukocytes via CD97 engagement. Herein, CDD5+ immune responses in the presence of TLR and Inflammasome ligand stimulation and eosinome incubation was studied. Fresh plasma and PBMCs of CHAPLE patients (N=7) before and after Eculizumab therapy (BT & AT, respectively) were obtained. PBMCs were stimulated with various ligands such as pL,C, LPS, R848, CpG ODNs, GAGMP Nigernic and BT and AT exosomes. Responses were assessed for IL-1β, IL-10, IL-10, IFNy and IFNα levels from cell supernatants. Results revealed that IL-8 and IP10 levels from plasma and stimulated BT cells were higher and AT exosomes were higher and ADP exosomes were secreted at lower levels than BT cells. The IFNα secretion BT and AT to PMA, R848 and GAGMP showed higher levels compared to healthy controls. Moreover, AT exosomes of patients induced substantially higher IFNy levels. IL-10 secretion in response to TR2L, 4 and 7 were found to be lower even AT but significantly increased compared to BT measurements. Furthermore, AT exosomes induced healthy BmC to secrete less IL-10 compared to BT exosomes. Spontaneous NENotic tendencies of patient neutrophils significantly subsided after Eculizumab therapy. Healthy or AT exosomes compared to BT exosomes induced much lesser NET formation from both healthy or AT neutrophils. Consequently, CDD5+ patients PBMCs regain healthy donor-like character following Eculizumab therapy.

WS.A6.01.03
A Th17 cell specific migration defect provides protection from EOE in DOCK8 deficient mice

A. S. Wilson1, H. Law1, C. B. Knobbe-Thomsen2, C. J. Kearney1, I. Olavar1, C. Binsfeld1, G. Burgio1, L. Starrs2, D. Brenner2, K. L. Randall2, A. Brüttlé2;
1The Australian National University, Canberra, Australia, 2Heinrich Heine University Düsseldorf, Düsseldorf, Germany, 3Peter MacCallum Cancer Centre, Melbourne, Australia.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 55

WS.A6.03.03
A Th17 cell specific migration defect provides protection from EAE in DOCK8 deficient mice

A. S. Wilson1, H. Law1, C. B. Knobbe-Thomsen2, C. J. Kearney1, I. Olavar1, C. Binsfeld1, G. Burgio1, L. Starrs2, D. Brenner2, K. L. Randall2, A. Brüttlé2;
1The Australian National University, Canberra, Australia, 2Heinrich Heine University Düsseldorf, Düsseldorf, Germany, 3Peter MacCallum Cancer Centre, Melbourne, Australia.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 55
WS.A6.01.04
Consequences of an IL2RA locus duplication in a very early onset inflammatory bowel disease patient
M. E. Joosse1,2, F. Charbit-Henriot3, R. C. Raatgever1, D. J. Lindenergh-Kortelve1, L. M. Costes1, S. Nugteren1, S. Veenbergen1, V. Malani4, J. K. Nowak5, M. Mearin4, J. C. Escher4, N. Cerf-Bensussan1, J. N. Samson6
1Erasmus MC, Rotterdam, Netherlands, 2Institute National de la Santé et de la Recherche Inserm, 3Institute Imagine and Université Paris-Descartes, Paris, France, 4Poznan University of Medical Sciences, Poznan, Poland, 5Leiden University Medical Center, Leiden, Netherlands.

Rare single genetic mutations can predispose to very early onset inflammatory bowel disease (VEO-IBD). Here, we identify a de novo duplication of the 10p15.1 chromosome region 10p15.1, in a 2-year-old patient presented with subcutaneous abscesses. VEO-IBD is very rare and has till now been described in only few patients. We report a pre-treatment STAT1-driven gene signature associated with response. Phenocopying this response-associated gene expression profile by rational pre-treatment of the tumour microenvironment sensitizes tumours to ICB.

WS.A6.01.05
Coincidence of a novel NFKB1 mutation with a Crohn-associated NOD2 rare variant in a patient may explain her profound common variable immunodeficiency (CVID) phenotype with unusually severe gastrointestinal manifestations
M. Martinez-Gallo1, R. Dieli-Crimi2, C. Franco-Jarava1, M. Antonlin1, L. Basco3, P. Paramonov4, A. Alvarez Fernandez4, X. Molero5, J. Velazquez5, A. Martin-Nalda6, R. Pujol-Besol7, R. Colabrun1
1Immunology Division, Hospital Universitari Vall d’Hebron, Barcelona, Spain, 2Department of Cell Biology, Physiology and Immunology, Autonomous University of Barcelona (UAB), Barcelona, Spain, 3ImmunoUnidad, Hospital U. de Vall d’Hebron, Barcelona, Spain, 4Area of Clinical and Molecular Genetics. Hospital U. Vall d’Hebron, Barcelona, Spain, 5Pneumology Department. Hospital U. Vall d’Hebron, Barcelona, Spain, 6Department of Digestive Diseases Hospital U. Vall d’Hebron, Barcelona, Spain, 7Pediatric Infectious Diseases and Immunodeficiencies Unit (UPIIP), Hospital Universitari Vall d’Hebron, Barcelona, Spain.

Monallelic loss-of-function mutations in NFKB1 have been recently recognized as one of the most frequent causes of common variable immunodeficiency (CVID). The profound phenotype and severe gastrointestinal manifestations in patients harboring NFKB1 deletions suggest that the NF-kB1 gene plays a critical role in human development. We report here a novel frameshift mutation in NFKB1 leading to a premature stop codon (p.Gly384Glu*48). Interestingly, we also found a rare missense variant in NOD2 known to be associated with Crohn’s disease (p.His532Arg). Our findings expand the spectrum of clinical disorders associated with NFKB1 and NOD2 loss-of-function mutations and support the hypothesis that NF-kB1 plays an important role in gastrointestinal health and disease.

WS.A6.01.06
Tetratricopeptide repeat domain 7A regulates haematopoietic stem cell functions by controlling the stress-induced response
C. Leveau1, M. El-Daher2, N. Cagnard3, A. Fischer4, G. de Saint Basile5, F. Sepulveda2
1Inserm U950, INSERM UMR 1363. Université Paris Cité, Paris, France, 2SFR Ncker, INSERM US24/CNRS UMS 3633, Paris, France, 3Assistance Publique-Hôpitaux de Paris, 4National Centre for Infections Diseases and Respiratory Medical and Pediatric Hematology Department, Paris, France, 5Collège de France, Paris, France, 6Austria.

The molecular machinery that regulates the balance between self-renewal and differentiation properties of hematopoietic stem cells (HSCs) has yet to be characterized in detail. We sought to determine the role of tetratricopeptide repeat domain 7A (Ttc7a) protein, a putative scaffold protein, in HSCs biological functions. We found that Ttc7a acts as a pro-apoptotic molecule in normal conditions and as a protector of HSCs from myeloablative stress. These findings shed new light on the role of IL-2 in intestinal homeostasis and direct further studies to examine the functional consequences of IL2RA genetic variation in IBD patients.

WS.B5.01.01
Immune checkpoints in anti-tumor therapy
S. Álvarez Fernández1, M. E. Joosse2, T. Hernandez Paredero2, A. Affananza Gonzalez2
1Hospital Universitario de La Princesa, Madrid, Spain, 2University Autónoma de Madrid, Madrid, Spain.

Tumor evasion frequently starts with the physiological immune system’s inability to mount an effective response to tumor invasion. The immune response can be reprogrammed in order to favor tumorigenesis. We analyzed the expression of the innate immune response in non-small cell lung cancer (NSCLC) to identify potential conditioning factors of response to treatment. For this, we have established a syngeneic mouse model of NSCLC marked with RGB lentivector multi-color cell tracking, to analyze clonal subpopulations in tumors treated with anti-PD1 or control antibodies. This has allowed us to track different clonal subpopulations derived from initial tumor and isolate resistant subsets by cell sorting, some of which were re-inoculated to analyze the behavior of each one individually. We have studied tumor progression, host immune response and gene expression profile in individual clonal subpopulations derived from anti-PD1- and control-treated NSCLC.

WS.B5.01.02
Rational therapeutic modulation of the tumour microenvironment sensitizes cancers to immune checkpoint blockade
1School of Medicine, Western University of Australia, Perth, Australia, 2National Centre for Asbestos Related Diseases, Perth, Australia, 3Telethon Kids Institute, Perth, WA, Australia, 4Harry Perkins Institute for Medical Research, Perth, Australia, 5School of Mathematics, University of Western Australia, Perth, Australia, 6Dept of Medical Oncology, Sir Charles Gairdner Hospital, Perth, Australia, 7Telethon Kids Institute, Perth, Australia, 8School of Biomedical Sciences, University of Western Australia, Perth, Australia.

Background: It is not well understood what molecular events contribute to an effective response to immune checkpoint blockade (ICB). Numerous combinations of drugs with ICB are currently being trialled with limited empirical preclinical evidence of efficacy. Predictive biomarkers and a rational approach to improve efficacy are therefore urgently needed.

Methods: Using inbred mouse strains inoculated bilaterally with monoclonal cancer cell lines, treatment with ICB results in a clear symmetric, yet dichotomous response, allowing to phenocopying this response-associated gene expression profile by rational pre-treatment of the tumour microenvironment sensitizes tumours to ICB.

Conclusion: We identified a pre-treatment STAT1-driven gene signature associated with response. Phenocopying this response-associated gene expression profile by rational pre-treatment of the tumour microenvironment sensitizes tumours to ICB.
Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer. Drug resistance and relapse are two major problems in ALL. Pre-B ALL cells express B-cell antigen receptor (CD19) and their immune response is mediated by natural killer (NK) and T cells. However, a significant minority of relapse pre-B ALL cells expresses lower CD19 expression. In vitro, drug-resistant pre-B ALL cells killed by NK cells and treatment with BAFF-R antibody, NK cells and EW-7197 may eradicate relapse and drug resistant pre-B ALL cancer cells.

**Conclusion:** We provide proof-of-principle that T cells with endogenous or genetically engineered specificity for HCC viral antigens can be targeted for functional genetic editing. We show that PD-1 knockdown enhances immediate tumour killing but is limited by compensatory engagement of alternative co-inhibitory and senescence programmes upon repetitive stimulation. Grants: EASL position paper fellowship, WT Senior Investigator Award.

**WS.B1.01.04**

**CD47-SIRPa checkpoint blockade involves kindlin3-dependent enhancement of CD11b/CD18 integrin affinity and cytotoxic synapse formation**

P. Boutilier, H. Matlani, M. van Houdt, P. Verkuijlen, K. Franket, T. W. Kuijpers, T. K. van den Berg;

Division of Infection and Immunity, UCL, London, United Kingdom; Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, Singapore; National University of Singapore, Singapore, Singapore; 3Navoarrabio-Biomedical Research Centre, IDoDNA, Pamplona, Spain; 4University College of London, London, United Kingdom; 5Department of Surgery and Interventional Science, UCL, London, United Kingdom; 6Institute of Medical and Dental Sciences, University of Liverpool, Liverpool, United Kingdom; 7Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, Singapore; 8Centre for Immunobiology, Bizzar Institut, Bart's and the London School of Medicine and Dentistry, QMUL, London, United Kingdom; 9Institute of Immunology and Transplantation, UCL, London, United Kingdom; 10Emerging Infectious Diseases Program, Duke-NUS Graduate Medical School, Singapore, Singapore.

**Introduction:** Checkpoint inhibitors and adoptive cell therapy provide promising options for treating solid cancers such as hepatitis B-related hepatocellular carcinoma but have limitations. We tested the potential to combine advantages of each approach, genetically re-programming T cells for specific viral/tumour antigens to overcome exhaustion by down-modulating the co-inhibitory receptor PD-1. Methods: We developed a novel lentiviral transduction protocol to achieve preferential targeting of endogenous low-frequency or TCR-redirected antigen-specific CDB T cells for sRNA knockdown of PD-1 and tested functional consequences for anti-tumour immunity in 3D and 2D cultures. Results: Antigen-specific CDB T cells transduced with LV-shPD-1 consistently had a marked reduction in PD-1 compared to those transduced with a control lentiviral vector. PD-1 could also be down-modulated on liver-resident or T cell receptor (TCR)-redirected T cells. PD-1 knockdown of human T cells rescued anti-tumour effector function and promoted killing of hepatoma cells. Conclusion: 3D microorganism recapitulating the pro-inflammatory PD-L1+ liver microenvironment evaluated in vivo. However, upon repetitive stimulation, PD-1 knockdown drove T cell senescence and induction of other co-inhibitory pathways. Conclusion: We provide proof-of-principle that T cells with endogenous or genetically engineered specificity for HCC viral antigens can be targeted for functional genetic editing. We show that PD-1 knockdown enhances immediate tumour killing but is limited by compensatory engagement of alternative co-inhibitory and senescence programmes upon repetitive stimulation. Grants: EASL position paper fellowship, WT Senior Investigator Award.

**WS.B1.01.05**

**HVEM as a new checkpoint blockade for cancer immunotherapy**

P. KC, S. Brunel, N. Auber, D. Olive, G. Marodon;

1Sorbonne Université - Centre d’immunologie et des maladies Infectieuses-Paris, UMR1135, Paris, France; 2Aix-Marseille Universités, Inserm, CNRS, CRCM, Institut Paoli-Calmettes, Marseille, France.

**Expression of the co-inhibitory molecule Herpes Virus Entry Mediator (HVEM) has been identified in a wide range of cancers and its expression level might be inversely correlated with patient survival.**

HVEM positive tumor cells could inhibit the immune response through co-inhibitory molecules B7-1 or PD-1, the main HVEM ligands expressed by human T lymphocytes. To release the anti-tumor immune response, a monoclonal antibody (mAb) targeting this immune checkpoint was evaluated in NSG mice, genetically deficient for T, B and NK cells. NSG mice grafted with PBMC and PC3, a human prostate cancer cell line expressing HVEM, showed a reduced tumor growth with anti-HVEM therapy whereas no effect was observed with another prostate cancer cell line not expressing HVEM. TILs had an increased proportion or proliferation of CD8+ T cells, suggesting that the mAb improved anti-tumor immunity. Altogether, these results indicate that CD47-SIRPa checkpoint blockade involves kindlin3-dependent enhancement of CD11b/CD18 integrin affinity and cytotoxic synapse formation.
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WS.B1.02.02
Identification of novel immunotherapeutic targets for colorectal cancer
D. Shinke1, H. McGuire1, T. Asshurt1, S. Clarke2, S. Byrne3, A. Charles2;
1Discipline of Pharmacology, The University of Sydney, Sydney, Australia, 2Discipline of Pathology, The University of Sydney, Sydney, Australia, 3Ramacell facility for Systems Biology, The University of Sydney, Sydney, Australia, 4Royal North Shore Hospital, Sydney, Australia, 5The Westmead Institute for Medical Research, Sydney, Australia, 6Discipline of Infectious Diseases and Immunology, The University of Sydney, Sydney, Australia.

An enhanced understanding of the immune-tumour interaction has led to significant clinical benefit in the use of immunotherapy in cancer. In colorectal cancer, however, a lack of understanding of the immune cell complexity means that the correct drug targets for exploitation remain elusive. Our aim was to investigate the immune phenotype and inflammatory signalling pathways in colorectal cancer patients undergoing chemotherapy to identify novel immunotherapeutic targets. Using a 35-marker mass cytometry panel, we quantified 7 major circulating immune cell types and over 20 subtypes in 10 advanced colorectal cancer patients and 9 healthy volunteers. We found significant differences in major B cell populations and sub-populations of T cells (including regulatory T cells, central memory T helper cells and effector memory cytotoxic T cells) between the patients and the healthy volunteers. Further interrogation of B cell sub-populations is underway. We also quantified nine phosphorylated intracellular signalling markers. At baseline, results show that pSTAT3 and p53A3 are significantly decreased in patients across the majority of immune cells whereas p53AT3 is increased. Results also show that phosphorylated markers, such as pERK, can be activated following a cycle of chemotherapy and remain activated throughout the therapy. Relationships between immune profiles and clinical outcomes are currently being explored. The use of mass cytometry has allowed the investigation of the immune profile of CRC patients and potential novel immunotherapeutic targets to be identified to improve clinical outcomes.

WS.B1.02.03
IL-18R is a novel checkpoint regulating anti-tumour and anti-viral activity of NK cells
M. Molgora1, E. Bonavita1, A. Ponsetta1, F. Riva1, M. Barbagallo1, S. Jallion1, B. Popovic1, G. Bernardini1, E. Magrin1, F. Gianni1, S. Zelenay1, J. Janjic1, A. Santoni1, C. Garland1, A. Mantovan1;
1Humanitas University, Pieve Emanuele, Italy, 2Humanitas Research Hospital, Rozzano, Italy, 3University of Rijeka, Rijeka, Croatia, 4Università di Roma “La Sapienza”, Roma, Italy, 5Cancer Research UK, Manchester, United Kingdom.

IL-18R is an Interleukin-1 receptor family member that acts as a negative regulator of IL-1 family receptor and TLR signaling. Both murine and human NK cells express high levels of IL-18R but its functional role in this cell type has not been described so far. Expression analysis showed that IL-18R was acquired during differentiation in human and murine NK cells. IL-18R deficiency in the mouse was associated with enhanced NK cell maturation and activation. IL-18, which is a key regulator of NK cell activities and can be targeted by IL-18R, was responsible for this phenotype. To assess the role of IL-18R in NK cells in pathology, we used models of MCA-induced lung metastasis, colon cancer-derived liver metastasis and DEN-induced hepatocellular carcinoma. The number and dimension of liver and lung metastasis and the liver disease severity were significantly reduced in Il18r−/− mice. The depletion of NK cells in these models totally abrogated the protection observed in Il18R−/− mice. Finally, we investigated the role of IL-18R in NK cell antiviral activity, in a model of MCMV infection. Il18r−/− mice controlled the virus more efficiently in the liver and the protection was associated with enhanced NK cell degranulation and IFN-γ production. The adoptive transfer of Il18r−/− NK cells conferred protection in both metastasis and viral infection models. Il-18R plays a non-redundant role in the regulation of NK cell development and effector functions by tuning IL-18-dependent activities. IL-18R therefore emerges as a crucial regulator of NK cell antitumour and antiviral potential.

WS.B1.02.04
Glycan modified vesicles as a targeted and personalized vaccination strategy for the induction of anti-tumor immunity
S. K. Hornevorts, D. A. Stoik, S. J. van Vliet, T. D. de Gruyj, A. A. van de Laar, Y. van Kooyk;
Vu university medical center, Amsterdam, Netherlands.

Effective immunotherapies should boost existing or elicit de novo, tumor-specific immune responses. Challenges in vaccine development are the choice of suitable antigens and targeting of these antigens to antigen presenting cells (APCs). We developed a new personalized vaccination approach, which encompasses apoptotic vesicles derived from the patients tumor and glycogen modulation, to allow targeting for APCs for the induction of tumor specific T cells. Apoptotic vesicles derived from the patients tumor contain both tumor associated antigens (TAAs) and neo-antigens, making them a potent source for vaccination. For the efficient targeting of the vesicles to APCs we modified their glyocalyx, resulting in the surface expression of high mannos glycan structures. The glycan are the natural ligand of the DC specific C-type lectin receptors DC-SIGN and Langerin, expressed on skin dermal DCs (dDCs) and Langerhans cells (LCs), respectively. Using ex vivo skin explants as a model for intradermal vaccination in humans, we are able to specifically target apoptotic vesicles to dDCs and LCs, thereby significantly increasing vesicle uptake, resulting in an enhanced antigen presentation to CD8+ T cells. In conclusion we developed a novel personalized vaccine strategy were we combine patient derived tumor vesicles with glycan modification to efficiently target (neo-) antigens to APCs, for the induction of antitumor immune responses.

WS.B1.02.05
Heme as a modulator of the therapeutic efficacy of an anti-cancer antibody
A. Tavares-Kanyavuz, A. Marey-Jarossay, S. Lacroix-Desmazes, J. Dimitrov;
Sorbonne University; INSERM UMR 5118, Centre de Recherche des Cordeliers, 75006 Paris, France.

Introduction: Polyreactive antibodies have potential to bind to multiple structurally unrelated antigens. Some apparently monoreactive antibodies like Rituximab can acquire polyreactivity post-translationally by contact with heme. Rituximab (anti-CD20) is used for the treatment of different B cell malignancies and among these diseases some can be accompanied by hemolysis. The main goal of this study is to characterize the effect of heme on therapeutic efficacy of anti CD20 antibodies. Materials and Methods: We performed in vitro studies with human B-cell lymphoma cell lines, to characterize the polyreactivity and cytotoxicity of Rituximab after heme-exposure. Then, we evaluated the impact of heme binding on the therapeutic activity of Rituximab using a murine B lymphoma model. Results: Rituximab acquires a large gain of reactivity after exposure to heme, while retaining the capacity to bind to its cognate antigen. Induction of polyreactivity improves therapeutic efficacy of Rituximab in vitro and in vivo. Conclusions: We documented an important role of the induced polyreactivity in the function of anti-CD20 antibodies. This phenomenon may occur in patients treated with Rituximab and could potentially have therapeutic repercussions. The induction of polyreactivity could represent a new axis for improvement of the therapeutic potential of Rituximab. The project is funded by ERC (StG-2015 CoBABAT) and Association for Cancer Research (ARC, France) grants.

WS.B1.02.06
Evaluation of a New Anti-Galectin 9 ImmunoTherapy Strategy in Pancreatic Cancers
A. Quillat1, R. Mustapha1, S. Renaud1, B. Duchêne1, C. De Schutter1, G. Herlin1, D. Moraïles1, I. Van Seuninger1, N. Jonckheere1, N. Delhem1;
1CNRS UMR 8161, Institut de Biologie de Lille, 59021 Lille Cedex, France, 2INSERM UMR837, Team S, 59021 Lille Cedex, France.

Background: We have previously described in an humanized mouse model of nasopharyngeal carcinomma that an anti-Galectin-9 (Gal-9) monoclonal antibody (mAb) is able to significantly limits tumor growth by specifically inhibiting the suppressive activity of human natural Tregs (Patent WD:WO2015185875). Herein, we propose to use this new specific active immunotherapy in a pancreatic cancer mouse model (KRAS12D12D), insofar as a high Treg prevalence has been described and correlated to the tumor progression of pancreatic cancer.

Methods and Results: Gal-9 expression was confirmed by immunohistochemistry on pancreas isolated from the KRAS12D12D mouse model. This Gal-9 expression level has been correlated to the progression of pre-cancerous lesions. Furthermore, an increase of Tregs prevalence has been observed in this transgenic model at a systemic and intratumoral level. Very interestingly, we also showed that (i) murine Tregs expressed Gal-9 (flow cytometry, immunofluorescence and western-blots), (ii) anti-Ga9 mAb neutralized the immunosuppression induced by recombinant murine Gal-9 (proliferation assay) and (iii) anti-GalA9 mAb neutralized the suppressive activity of murine Tregs (MLR assay). Further investigations performed on four human pancreatic cancer cell lines (Capan-1, Panc-1, G12D) have also confirmed the Gal-9 expression at a genomic (RT-qPCR), proteomic (Immunofluorescence, Western-blots and flow cytometry) and secreting (ELISA) level.

Conclusion: Our preliminary results suggest that the use of an anti-Gal-9 mAb could be considered as a new anti-tumour immunotherapy targeting Tregs in the pancreatic cancer.

58
WS.B1.03 Genetically engineered TCR for immunotherapy

WS.B1.03.01 Development and characterization of novel anti-GD2 target modules for retargeting of Universal CAR T cells toward GD2 expressing tumors

N. Mitwasi1, A. Feldmann1, R. Bergmann1, N. Berndt1, C. Rössig1, M. Bachmann1,2,3,4,5
1Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rosendorf (HZDR), Dresden, Germany, 2Department of Pediatric Hematology and Oncology, Münster University Hospital (UKM), Münster, Germany, 3Tumor Immunology, University Cancer Center (UCC) Carl Gustav Carus TU Dresden, Dresden, Germany, 4German Cancer Research Center (DKFZ), Heidelberg, Germany.

Although chimeric antigen receptor (CAR) engineered T cells demonstrated promising therapeutic effect against cancer, they are still associated with adverse side effects which could be life threatening in some cases. Therefore, in our group we have developed a switchable universal CAR T cell platform “UniCAR”, which can be repeatedly switched on and off. This system consists of CAR T cells that cannot bind tumor antigens directly but instead they are redirected with a target module (TM). Such TMs are mainly composed of an epitope on one side, which is recognized by the UniCAR T cells, and on the other side a tumor antigen binding domain. Once the TM is eliminated, the UniCAR T cells are no more activated. Disialoganglioside GD2 was shown previously to be a very promising target for several tumors such as neuroblastoma and Ewing’s sarcomas. Therefore, anti-GD2 TMs were developed and evaluated regarding their functionality. They were shown to be functional in activating the UniCARs to secrete important pro-inflammatory cytokines and to kill GD2+ tumor cells both in vivo and in vitro. To further characterize the anti-GD2 TM with PET imaging, it was labeled with radioactive Cu64. The TM showed a specific enrichment at the site of the GD2+ tumor. This was eliminated through kidney function within half an hour due to its small size. Such short half-life, provide the UniCAR system with the fast safety switch in case any complications occurred in patients treated with the UniCAR T cells.

WS.B1.03.02 Functional comparison of CARs targeting CD20 with a TCR directed against a CD20 derived peptide

T. L. A. Wachsmann1, L. Jahn1, E. van Dijest1, J. Leusen1, J. Kuball1, J. Falkenberg1, M. Heemskerk1
1UMC, Leiden, Netherlands, 2UMC Utrecht, Utrecht, Netherlands.

With the rise of chimeric antigen receptor (CAR) T-cells, the role & potential of T-cell receptors (TCRs) targeting surface antigen derived peptides for gene transfer therapy needs to be reevaluated. While the non-HLA-restricted CAR T-cells have demonstrated remarkable clinical efficacy in hematological malignancies, severe toxicity in responders and non-responders in a subset of patients remains major challenges. We hypothesize that TCR-transduced T-cells targeting tumor surface antigen derived peptides pose a valuable alternative to CAR T-cells by maintaining a comparable efficacy while offering a more tolerable toxicity profile, alongside higher resistance to activation induced cell death and exhaustion.

Our group has previously identified a high affinity TCR targeting a CD20 derived peptide presented in the context of HLA-A2. Using a panel of human acute lymphoblastic leukemia cells with different levels of CD20 expression, we aim to functionally compare our TCR with four 4-1BB-CD3z second generation CARs differing in their CD20 recognition domain. Primary endpoints are functionality (killing capacity & cytokine profiling), resilience, proliferative capacity and maintenance of functionality after antigen challenge. Preliminary in vitro studies reveal markedly elevated cytokine production alongside indications of accelerated killing kinetics in the CAR T-cells as opposed to the TCR transduced T-cells in a CD20 expression sensitive manner. However, this appears to trade off against activation induced cell death and severe impairment of proliferative capacity in response to high levels of antigen exposure in the TCR cells. Ultimately, our experimental framework provides a rationale for the future direction of antigen receptor design.

WS.B1.03.03 Impaired Early Downstream Signaling Blunts Antigen Sensitivity of CAR-T-cells

V. Gudipati1, J. Rydzek1, I. Perez2, S. Königberger1, H. Stockinger1, M. Hudecek1, J. Huppa1
1Medical University of Vienna, Vienna, Austria, 2Universitätsklinikum Würzburg, Würzburg, Germany.

Adoptive immunotherapy employing chimeric antigen receptor (CAR)-modified T-cells has given rise to new hope in oncology as an effective treatment regime for advanced malignancies. While high rates of complete remission after CAR T-cell therapy can be obtained in patients with B cell malignancies, relapse may occur in significant number of patients in the settings of other hematopoietic cell lineages. Rational design of CARs is essential for the development of CARs targeting different cell types of different origin and function in order to increase the efficacy of CAR-T cell therapies. Here, we show that antigen recognition triggers a complex signaling cascade involving early lymphocyte activation molecules and how antigen-engagement triggers activation. To gain a deeper insight into the mechanisms of CAR-induced activation and the development of the CAR immunological synapse, we employed total internal reflection fluorescence (TIRF) microscopy. We found the sensitivity of CAR-T cells towards antigens is reduced by 500 times when compared to T-cell antigen receptor-mediated detection of nominal peptide/MHC complexes. While CAR antigen binding was efficient, receptor proximal signalling was significantly attenuated due to reduced recruitment of the tyrosine kinase ZAP70 at ligated CARs. At limiting antigen densities absence of adhesion molecule ICAM1 significantly affects CAR T-cell mediated cytotoxicity indicating that blunted CAR signalling leads to attenuated activation of the integrin LFA-1, thereby compromising cell adhesion. Our findings expose fundamental limitations of current one-dimensional CAR designs that has to be overcome for personalized cancer treatment. Furthermore, our findings highlight unique strengths of live molecular imaging for preclinical CAR-development.

WS.B1.03.04 Antitumor activity by TEGs: alpha/beta T cells engineered to express a defined gamma/delta TCR in a 3D bone marrow niche model of multiple myeloma

T. Straetemans1, M. Braham1, T. Aarts-Riemens1, J. Albasi1, M. Minnema1, Z. Sebestyen1, J. Kuball1
1Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht, Netherlands, 2Department of Orthopaedics, University Medical Center Utrecht, Utrecht, Netherlands.

y8T cells mediate cancer immune surveillance by sensing metabolic changes of malignant cells via their y8TCR. This concept led to the development of next generation CAR T cells, so-called in CSV: y8T cells Engineered to express a defined y8TCR. A particular y8deltaTCR, has been selected as candidate for clinical testing (TEG001). TEG001 cells showed a strong and broad recognition of hematopoietic malignancies and are able to differentiate between healthy and leukemic stem cells. Important for the therapeutic success of immune therapy concepts such as TEGs is a better understanding of the interplay between malignant, stromal and immune cells in the tumor microenvironment. To this end a 3D model was established that allowed engraftment of primary multiple myeloma (MM) cells within a humanized bone marrow niche...TEG001 cells, but not mock engineered T cells, migrated into the 3D structure and exerted a killing response towards the tumor cells but not towards the stromal cells. This cognate recognition was associated with the differential production of chemokines, cytokines and inhibitory molecules. Amongst others, TEG001 cells induced CCL1 secretion, but also the secretion of IL-6 and GM-CSF, reported to be involved in cytokine release syndrome. Soluble antibody that targets GM-CSF and IL-6 significantly reduced CCL1 and GM-CSF secretion in the 3D model. These data suggest that TEG therapy activates the immune system to recognize and eliminate myeloma cells while reducing immune system toxicity.

WS.B1.03.05 Engineering antigen-specific Natural Killer cells against the melanoma-associated antigen tyrosinase via TCR gene transfer

A. Parlar1, C. Pamukcu1, E. C. Sayitoglu2, A. Georgoudaki3, D. Ozakcan4, M. Asar1, M. Orobok1, P. Zahedimaram1,2, L. Kromzoda1, E. Alıcı1, B. Erman1, A. D. Dursu1, T. Satlı1
1Nanotechnology Research and Application Center, Sabancı University, Istanbul, Turkey, 2Faculty of Engineering and Natural Sciences, Sabancı University, Istanbul, Turkey, 3NSU Cell Therapy Institute, Nova Southeastern University, Florida, United States, 4Center for Hematogen and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden.

While genetic modification of cytotoxic T lymphocytes (CTLs) to target intracellular antigens or using Chimeric Antigen Receptors (CARs) to target cell surface antigens are commonly investigated; for Natural Killer (NK) cells, CARs have so far been the only practical method of antigen-specific retargeting, TCR gene therapy can supply large populations of CTLs genetically modified to express a specific TCR, but the mispairing of endogenous and genetically transferred TCR subunits constitutes a bottleneck in the development of safe therapies. In order to overcome this obstacle and open the realm of intracellular antigens to targeting by NK cells, we propose to use NK cells for TCR gene transfer. Our group has recently shown that both the TCR a/b heterodimer, nor the CD3 subunits had the capacity to transport to the cell surface alone but could only form a stable complex when all components were present. The introduction of a functional TCR complex to NK cells enabled antigen-specific and MHC-restricted triggering of effector functions against tyrosinase expressing tumor cells both in vitro and in vivo. This strategy not only opens the realm of intracellular antigens to targeting by NK cells but also provides a definite solution for the mispairing problem observed in TCR gene therapy.
Tumor-Resident γδ T Cells Are Cancer Immunosurveillance Sensors


1. Department of Hematology and Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht, Netherlands, 2. Institute of Medical Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal, 3. Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.

γδ T cells mediate cancer immune surveillance by sensing metabolic changes of malignant leukemic blasts and not their healthy counterpart via their γδ T cell receptor (TCR). This concept led to the development of next generation CAR T cells, so-called TEGs: γδT cells Engineered to express a defined γδTCR. A particular γδOTCR, isolated from “clone S”, has been selected as the candidate for clinical testing (TEG001). TEG001 cells showed a strong and broad recognition of hematological malignancies against both cell lines and primary AML.

In order to evaluate the biodistribution and safety profile of TEGs we developed a patient-derived xenograft (PD-X) in vivo model by establishing primary malignant AML blasts, which tested positive in an in vitro assay for recognition by TEGs, in NGS mice. In addition, healthy stem cells from human cord-blood were in parallel engrafted in a separate set of NGS mice. After engraftment, TEGs were infused and mice followed for additional 50 days. While engrafted primary AML blasts were no longer detectable in the peripheral blood at the end of the study period, all healthy hematopoietic cellular compartments remained unharmed. Within the limitations of humanized PD-X models, TEGs target acute myeloid leukemia but do neither interfere with engraftment of hematopoietic progenitors nor harm matured subsets of the hematopoiesis. In addition, no additional signs of off-target toxicity were observed in mice. TEGs are a promising addition to the currently available immune therapeutic strategies as they target cancer as a metabolic disorder.

Harnessing Pre-Existing Antiviral Immunity to Treat Solid Tumors

N. Cuburu, R. Kim, S. M. Pantejov, C. D. Thompson, D. R. Lowry, J. T. Schiller

1. National Cancer Institute, NIH, Bethesda, United States, 2. National Institute of Allergy and Infectious Diseases, NIH, Bethesda, United States.

Human cytomegalovirus is highly prevalent in humans with polyfunctional T cell responses expanding with age. We questioned whether redirecting pre-existing anti-cytomegalovirus (anti-CMV) T cells into solid tumor could arrest tumor growth, induce epitope spreading, and confer long-term anti-tumor immunity. Persistently infected mice with murine cytomegalovirus (mCMV) were challenged with TC-1 tumor cells expressing human papillomavirus (HPV) E6 and E7 oncogenes. In vivo transduction of TC-1 tumors with a viral vector expressing MCMV antigens or intratumoral injection of peptide mCMV epitopes with a TLR3 agonist (poly I:C) caused the expansion of mCMV-specific CD8+ and CD4+ T cells. Using PanCancer Immune profiling panel (Nanostring) we show that intratumoral injection of mCMV peptide epitopes induced massive modifications of the tumor innate and adaptive immune environment. Intratumoral injection of mCMV CD4 peptide epitopes with poly I:C promoted more significant expansion of T cells than mCMV CD8 peptides. Sequential administration of CD4 and CD8 mCMV epitopes together with poly I:C was the best protocol to eradicate pre-existing tumor of 5 to 10 mm diameter, and rechallenge experiments showed antitumor immunity up to 4 months after the last treatment. Our results provide a proof of concept to design “antigen-agnostic” intratumoral therapies based on pre-existing antiviral T cells. Such approach could change tumor immune microenvironment, induced epitope spreading, and conferred long-term anti-tumor immunity. These findings prompt further evaluation in other spontaneous tumor models and provide a model to decipher the mechanisms of epitope spreading notably to investigate CD4 T cell help.

Ephemeral immune control of cytomegalovirus infection by T-cells recognizing a single viral epitope

F. Mbiu, I. Barke, Z. Chaudhry, L. Cicin-Sain; Helmholtz Center for Infection Research (HZI), Braunschweig, Germany.

The cytotoxic T cells play an important role in the control of viral infections. T-cell based adoptive immunotherapy using antigen specific cells is explored as a treatment option for CMV disease. However, therapeutic success varies among individual recipients and the underlying reasons remain unclear and unpredictable. To understand the minimal requirements for adoptive immunotherapy of CMV disease, we generated recombinant murine CMVs expressing the immunodominant epitopes SSIEFARL or KCRMNIRDQVL. We infected TCR transgenic mice on a RAG2-/- background, recognizing only these epitopes, and followed their survival upon infection. While wild-type virus rapidly killed the mice, recombinant viruses expressing the corresponding epitope were controlled. However, the immune protection was transient, because the mice succumbed by 6-8 weeks post infection. To test if poor viral control was caused by T-cell exhaustion, we analyzed their phenotype. While we observed an accrual of exhausted T cells (PD1+, Eomes-) at times of death, these increases were modest. Most T cells remained PD1-negative and retained functionality in ex vivo assays. Alternatively, viral escape of immune recognition could have explained the phenomenon. Therefore, we isolated MCMV genomes from organs of infected mice at time of death and sequenced them. We identified epitope deleterious mutations in the vast majority of viral genomes, which were sufficient to prevent T-cell recognition of infected cells. Our data argue that T-cell immunotherapy against one immunodominant epitope may provide transient protection against CMV, but also drive immune escape. Therefore, our data indicate that optimal T-cell protection may require targeting multiple epitopes.

Autologous neo-antigen-specific T cell responses in low mutation burden colorectal cancers

J. van den Bulk, D. Ruan, M. Visser, M. Asselstijn, R. van der Breggen, K. Peeters, S. van der Burg, M. E. Verdegaal, N. de Miranda; LUMC, Leiden, Netherlands.

Innovative treatment options are required to improve cure rates in advanced colorectal cancer patients. Immune checkpoint blockade therapy (anti-PD-1) was shown to be effective in colorectal cancers with high mutation burden (e.g. mismatch repair deficient) as anti-tumour reactivity is largely explained by the recognition of somatically mutated antigens (neo-antigens). No immunotherapeutic strategies are currently available for patients diagnosed with low mutation burden CRC, while they could greatly benefit from the induction of immune responses. We hypothesized that if autologous neo-antigen-reactive T cells are present in such patients, they might benefit from specific immunotherapeutic interventions that stimulate neo-antigen recognition. In order to detect neo-antigens, whole exome and RNA next-generation sequencing were performed in cancer and healthy tissues from colorectal cancer patients. Corresponding peptides were synthesized and tested for their ability to induce immune cell activation in lymphocytes isolated from the tumour tissue and from peripheral blood. Nearest-car ответ T cells were cultured from 5 out of 10 CRC cell lines and 6 out of 6 healthy donors. Most T cells were expressed in the tumour tissue from a CRC patient. In conclusion, we developed a neo-antigen screening pipeline to unlock the immunogenic potential of colorectal cancers with low mutation burden. We have detected a relatively high number of neo-antigens that are recognized by autologous T cells in a mismatch repair proficient, low mutation burden CRC patient. This finding supports the widespread potential of the employment to employ neo-antigen-targeted therapies to improve the treatment of colorectal cancer patients.
WORKSHOPS

WS.B1.04.05

Abstract of tumor associated antigen specific T-cells restricted to self-HLA alleles is of sufficient avidity to recognize overexpressed endogenously processed antigen

1Leiden University Medical Center, Leiden, Netherlands, 2Juno Therapeutics, Göttingen, Germany.

Tumor associated antigens (TAA) are proposed as targets for graft versus leukemia effect after HLA-matched allogeneic stem cell transplantation. As TAA are self-antigens, high avidity TAA-specific T-cells are thought to be eliminated from the T-cell repertoire by thymic selection. In this study, we investigated whether TAA-specific T-cells with sufficient avidity to recognize overexpressed endogenously processed antigen in self-HLA can be found in healthy donors. T-cells directed against TAA peptides NY-eso-1-SLAA*02:01, WIL-1-SLAA*02:01, Prostate-3-VLQA*02:01 and PRAME-VLDA*02:01 were isolated from HLA-A*02:01+ donors using MHC-I-Streptamers. Generated tetramers+CD8+ T-cell clones were classified based on the minimal concentration of peptide exogenously loaded on TAP-deficient T2-cells needed for cytotoxic production. To analyze recognition of overexpressed endogenously processed antigen, high-potential clones were tested against HLA-A*02:01+ ENU-LEC transduced with the full corresponding TAA sequence. >800 tetramer+CD8+ TAA-specific clones were isolated from 18 donors. T-cell receptor Vbeta-family analysis revealed a minimal number of unique clones: 14 NY-eso-1-SLAA*02:01, 30 WIL-1-SLAA*02:01, 14 RHAMM-VLAA*02:01 and 8 Prostate-3-VLQA*02:01 and 14 PRAME-VLDA*02:01 clones. The functional screening revealed 27 non-functional clones, 16 low-potential clones (activation threshold ≤10^-6M peptide) and 37 high-potential clones (activation threshold ≤10^-7M peptide). Of the high-potential clones, only 2 NY-eso-1-SLAA*02:01, 7 WIL-1-SLAA*02:01 and 5 PRAME-VLDA*02:01 clones showed recognition of overexpressed endogenously processed antigen. These results illustrate that self-HLA restricted TAA-specific T-cells can be easily isolated from donor PBMC, but that only a minority of T-cells are capable of recognizing overexpressed endogenously processed antigen. Classification of functional TAA-specific T-cells by only high tetramer staining and peptide specificity leads to overestimation of avidity.

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Cross-reactive CD8+ T cell receptor clonotypes elicited by influenza epitope variants are drawn from a narrow repertoire distinct from non-cross-reactive receptors

P. G. Thomas, P. Dash, P. Bradley, A. Williams, S. Duan;
St. Jude Children's Research Hospital, Memphis, United States.

T cells specific for one peptide-MHC (pMHC) epitope can occasionally cross-react with closely related pMHC, such as those containing single amino acid mutations. What determines the extent of cross-reactivity has not been well-characterized. To define the "rules" of cross-reactivity, we chose two variants of the influenza Dp44, NP-epitope with a single residue change, [NP-]TBA and [NP-NLA], that are known to elicit cross-reactive responses to NP-wt. We analyzed the paired a)TCR repertoire of cross-reactive cells from mice infected with either of the variant viruses singly or in competition with a prime-challenge model. Each combination elicited highly variable degrees of cross-reactivity, ranging from 20% (TBA elicited cells reacting to wt epitope) to 90% (NLA-elicited cells reacting to wt epitope). Most strikingly, regardless of the magnitude of the cross-reactivity, cross-reactive cells between each NP variant displayed an extremely narrow TCR repertoire diversity, often dominated by a single clonotype, while the non-cross-reactive cells had markedly higher diversity in their TCR repertoire. Importantly, many of these cross-reactive receptors did not appear to be the dominant receptors responding to the eliciting epitope (i.e. they had a higher TCRdiversity). Our results indicate that cross-reactivity is mediated by a rare subset of TCRs that are not representative of the typical responses elicited by an epitope. This work has implications for tumor immunity, as it provides insight into how to avoid self-reactivity, and vaccine design, as repeated immunizations with distinct epitopes is likely to select for extremely narrow, cross-reactive repertoires.

WS.B1.05.01

Antitumor immunology

dissecting the immune heterogeneity of neuroblastoma microenvironment in murine models to develop novel therapeutic strategies for high-risk neuroblastoma patients

Ospedale Pediatrico Bambino Gesù, Rome, Italy.

Introduction The presence of tumor-infiltrating T cells (TILs) and the absence of immunosuppressive elements have been associated with favorable prognosis of high-risk neuroblastoma (NB) patients. Recently, a subset of intratumoral dendritic cells (iDC) has been found crucial for antitumor-induced anticancer immune responses suggesting that the TILs of NB patients have the capacity to improve NB therapy. However, the immune infiltrate in NB murine models treated with chemotherapeutic drugs in combination with immune checkpoint blocking antibodies. Methods Luciferase-expressing NB cell lines derived from spontaneous tumors arising in the TH-MYCN transgenic mice were injected subcutaneously or orthotopically in syngeneic mice. Mice bearing established tumors were sacrificed and TILs were analysed by flow cytometry. The crosstalk between immune and tumor cells was evaluated in drugs-treated NB spheroids co-cultured with tumor-infiltrating CD4+ T cells. Results Tumor microenvironment was characterized by a consistent number of CD45+ immune cells, including T cells, NK cells, NKT cells, macrophages, neutrophils and iDC. The orthotopic model showed a more aggressive phenotype than the subcutaneous model, resulting in the development of tumor with an immunosuppressive microenvironment predominantly infiltrated by macrophages, myeloid-derived suppressor cells and T regulatory cells. Treatment with anti-infiltrating T cells resulted in a reduced diameter of spheroid and an increased recruitment of T cells. In vivo experiments are currently underway to evaluate the TILs in tumor-bearing mice treated with anti-infiltrating-derived drugs and immune checkpoint blocking antibodies. Overall, we provide insights for the study of a novel immunotherapeutic approach in NB.

WS.B1.05.02

Deficiency of host CD96 and PD-1 or TIGIT enhances tumor immunity without significantly compromising immune homeostasis

H. Harjumaa1,2, J. S. Blake1, E. Ahern3,4, V. Allen3, J. Liu1, Y. Yuan1,2, Lukuty1, K. Takeyoshi1, A. Roman Aguilera1, C. Guillery2, D. Mittal3,2, K. Y. Liu, W. C. Dougall1, M. J. Smyth1,2, M. W. Teng1,2;
1QIMR Berghofer Medical Research Institute, Brisbane, Australia, 2The University of Queensland, Brisbane, Australia, 3Royal Brisbane and Women’s Hospital, Brisbane, Australia, 4Suntundo University, Tokyo, Japan.

Multiple non-redundant immunosuppressive pathways co-exist in the tumor microenvironment and their co-targeting can increase clinical responses. Indeed, concurrent blockade of CTLA-4 and PD-1 in patients with advanced melanoma increased clinical responses over monotherapy although the frequency and severity of immune-related adverse events also increased. Nevertheless, a substantial number of patients still display an intact resistance phenotype and are unresponsive to current approved immunotherapies even when utilized in combination. In this study, we generated Pdcdl1CD96 and Tigit-CD96 mice to investigate how loss of CD96 in combination with PD-1 or TIGIT impacts on immune homeostasis and hence the potential of inducing immune related toxicities following co-targeting of these pairs of receptors. The ability of Pdcdl1CD96 and Tigit-CD96 mice to suppress primary tumor growth was also assessed using the MC38 colon carcinoma and SM1W1T Braf-mutated melanoma tumor models. Both Pdcdl1-CD96 mice showed no overt perturbations in immune homeostasis over what was previously reported with Pdcdl1 or Tigit mice when aged for 22 months. Interestingly, increased suppression of subcutaneous tumor growth and complete responses was seen in Pdcdl1-CD96 mice compared to Pdcdl1 or CD96 mice depending upon the tumor model. This enhanced anti-tumor efficacy of Pdcdl1-CD96 mice appeared to be due to favorable changes in the ratio of CD8+ T cells to Tregs or CD11c+Gr-1+ myeloid cells in the tumor microenvironment. Co-targeting CD96 and PD-1 may increase anti-tumor responses targeting CD96-1 and potentially not induce serious immune-related toxicities if used in humans. This study has implications for tumor immunity, as it provides insight into how to avoid self-reactivity, and vaccine design, as repeated immunizations with distinct epitopes is likely to select for extremely narrow, cross-reactive repertoires.

WS.B1.05.03

Prostate Biograft Reprograms Tumor Microenvironment in Pancreatic Cancer

1INSERM 1052, Lyon, France, 2INSERM 1058, Marseille, France, 3INSERM 1037, Toulouse, France, 4INSERM 1052, Lyon, France, 5University of Turin, Turin, Italy, 6KIST, Seoul, Korea, Republic of, 7Hospices Civils Lyon, Lyon, France, 8Centre Leon Bhard, Lyon, France, 9ENS Lyon, Lyon, France, 10CRIL, INSERM 1052, Lyon, France.

Pancreatic cancer is associated with an abundant inflammatory role leading to immune escape and tumor growth. This massive stroma drives the immune escape in the tumor. We identified this biograft as a potential tumor microenvironment as a key actor of the immune paracrine interactions mechanism that drives pancreatic cancer. We performed studies with p48-Cre;KrasG12D; pdk1-Cre;KrasG12D; pdk2-Cre;KrasG12D; pdk3-/- mice and tissues from patients with PDA. Some transgenic mice were given injections of antip-biograft depleting antibody (bAb). Tumor growth and metastatic growth of the biograft as modifications in the activation of local immune cells were analyzed by flow cytometry, immunohistochemistry, immunofluorescence and stiffness by atomic force microscopy. We found that biograft is highly produced by cancer associated Fibroblasts in the stroma of Human and mice. This protein acts directly on tumor specific CD8+ T cells and F4/80 macrophages. Depleting biograft in vivo reduced tumor growth by enhancing the number of activated CD8+ T cells within the tumor and subsequent apoptotic tumor cells. More importantly, we found that targeting biograft in established lesions increased immune-mediated tumor clearance by releasing the tissue tension and functionally reprogramming F4/80 macrophages in the tumor microenvironment. Our findings present biograft as a novel immunotherapeutic target in pancreatic cancer.
CD1-restricted T cells specific could play the central role in colorectal cancer immune surveillance, where self-antigens are the targets of immune responses. We showed that primary colorectal adenocarcinoma (A0L) and B-lymphoblastic (B-ALL) leukemia blasts express CD1c and are recognized by a group of CD1c self-reactive T cells specific for methyl-lyso phosphatidylcholine (mLPs), a novel class of self-antigen that accumulate in malignant cells. mLPA specific T cells can kill and control leukemia growth in vitro and in vivo. These findings point to CD1c and self-antigens as new potential targets for leukemia immunotherapy. The little polymorphism of CD1c molecules and their selective expression on mature leukocyes are indeed highly attractive for adoptive cell therapy (ACT) with such T cells in the context of stem cell transplantation for hematological malignancies. To assess the feasibility of ACT for acute leukemia with mLPA-specific T cells, we generated a library of lentiviral vectors encoding a panel of human mLPA-specific TCRs. Upon TCR transduction, either Jurkat T cells or human primary T cells were specifically retargeted against CD1c-expressing malignant targets in vitro, highlighting a lead mLPA-specific TCR suitable for adoptive immunotherapy. Primary T cells transduced with this TCR killed CD1c-expressing malignant targets in vitro and significantly delayed leukemia progression in NSG mice. Tgain further insight into the role of type 1 conventional dendritic cells (cDC1) in anti-tumor strategies, we constructed a CAM assay with fertilized chicken eggs as a model. The CAM acts as the growth surface for development of solid tumors using cancer cell lines. This method allowed us to study tumor growth characteristics in a 3D physiological model along with angiogenesis and differentiation potential of the tumor. We observed higher angiogenesis potential of the CAM3 COSMC knockout cell lines, suggesting a role for Tn antigen. In addition, we have modified the CAM assay to include monocytomas in the tumor to study g lancycogenase-2 activity restricts tumor growth and predicts cancer patient outcome

It is essential to develop new clinical strategies to treat the natural anti-tumor CD8+ T cell responses, or to induce de novo protective immunity in cancer patients. Here, we aim at analyzing what type 1 conventional dendritic cells (CD1c) functions can be manipulated to improve immunotherapies currently applied to cancer. CD1c express in tumor antigen cross-presentation, and are strong inducers of CD8+ T cell responses in many experimental settings. In human, tumor infiltrating CD1c1 are associated with a good prognosis in many solid cancers. Therefore, the protective functions of CD1C need to be described both in context of immunotherapies against cancers and during natural tumor immunosurveillance to tumor. We have generated unique mouse models, which allow either a constitutive or a conditional depletion of all cDC1 through the body. Using these models, we found that cDC1 are dispensable for tumor control at the time of immunotherapy administration (ACT or checkpoint blockers) in thymoma, melanoma and prostate cancer models. Nevertheless, cDC1 are critical to tumor immunosurveillance in a model of breast cancer that is naturally controlled when orthotopically engrafted in mice. In this last setting, CD1c protective functions depend on anti-tumor CD8+ T cells and their infiltration into the tumor. Although our work puts in perspective recent findings about the involvement of CD1c in immunotherapies against cancer, it will nonetheless help in defining new ways to mobilize CD1c functions to improve immunotherapies currently applied in clinics to patients.

Generation of Memory Stem T cells specific for tumor antigens and resistant to inhibitory signals by genome editing

It is essential to develop new clinical strategies to treat the natural anti-tumor CD8+ T cell responses, or to induce de novo protective immunity in cancer patients. Here, we aim at analyzing what type 1 conventional dendritic cells (CD1c) functions can be manipulated to improve immunotherapies currently applied to cancer. CD1c express in tumor antigen cross-presentation, and are strong inducers of CD8+ T cell responses in many experimental settings. In human, tumor infiltrating CD1c1 are associated with a good prognosis in many solid cancers. Therefore, the protective functions of CD1C need to be described both in context of immunotherapies against cancers and during natural tumor immunosurveillance to tumor. We have generated unique mouse models, which allow either a constitutive or a conditional depletion of all cDC1 through the body. Using these models, we found quite unexpectedly, that cDC1 are dispensable for tumor control at the time of immunotherapy administration (ACT or checkpoint blockers) in thymoma, melanoma and prostate cancer models. Nevertheless, cDC1 are critical to tumor immunosurveillance in a model of breast cancer that is naturally controlled when orthotopically engrafted in mice. In this last setting, CD1c protective functions depend on anti-tumor CD8+ T cells and their infiltration into the tumor. Although our work puts in perspective recent findings about the involvement of CD1c in immunotherapies against cancer, it will nonetheless help in defining new ways to mobilize CD1c functions to improve immunotherapies currently applied in clinics to patients.
WORKSHOPS

WS.B1.06.03
A CD3exCD19 bispecific DART™ molecule induces T-cell mediated killing of CLL cells in vitro


Chronic lymphocytic leukemia (CLL) is an incurable B-cell malignancy, associated with severe T-cell dysfunction. Recently, CD3exCD19 bispecific antibodies have been successfully applied in lymphoblastic leukemia, but efficacy in CLL has not been thoroughly assessed. We investigated whether CLL cells can be targeted by CD3exCD19 bispecific DART™ (also known as MGD011) therapy and if this overcomes T-cell dysfunction in CLL.

We found that MGD011 redirects healthy T-cells to kill CD19+ lymphoma cell lines, and primary CLL cells. Clinical responses seen in current therapies are highly dependent on specific prognostic factors (unmutated IgVH, high-risk cytogenetics, and chemoresistance). However, MGD011, killing of CLL cells could be achieved at comparable levels among these different prognostic subgroups.

Next, we assessed the responses of CLL-derived T-cells upon therapy with MGD011. When autologous CLL cells were used as targets, addition of MG011 resulted in robust activation of both CD8+ and CD4+ cells, measured by CD25 upregulation, proliferation, TNFα and IFNγ production and degranulation. CLL-derived T-cells (CD4 vs CD8 vs combined) were able to kill CD19+ cell lines (average specific lysis at a 1:1 T:E ratio of 85%). Killing of autologous CLL by single CD4 or CD8 cells was remarkably lower but 1:1 combination of CD4 and CD8 resulted in enhanced killing capacity (21%, 25% and 45% for single CD4, single CD8 and combined respectively).

Together, this indicates that even high-risk CLL cells can be killed using MGD011. Nevertheless, we are currently investigating means to further improve the T-cell mediated lysis of CLL cells.

WS.B1.06.04
MDM2-targeting as a new Natural Killer cell-based immunotherapy of neuroblastoma

I. Veneziani, E. Ferretti, D. Fruci, L. Moretti, V. Pastore, L. Locati, L. Cigaldi; 1Department of Pediatric Hematology and Oncology, Bambino Gesù Children’s Hospital, Rome, Italy, 2Laboratory of Oncology, Gianna Gaslini Institute, Genoa, Italy, 3Immunology Research Area, Bambino Gesù Children’s Hospital, Rome, Italy.

Neuroblastoma (NB) is the most common extracranial solid tumor occurring in childhood. Amplification of the MYCN oncogene is associated with poor prognosis. Downregulation of ligands on tumor cells recognized by Natural Killer (NK) cell-activating receptors, involved in tumor cell recognition and lysis, may contribute to tumor progression and relapse. We demonstrated that MYCN expression is inversely correlated with ligands recognized by NK and T cells, leading to functional impairment of NK cells, thus rendering them more susceptible to NK cell-mediated killing and in mediated killing, and in contrast to that, B16 F10 tumor cells are more resistant to NK cell-mediated killing.

We explored a molecular mechanism of non-classical physicochemical communication deployed by high glycolytic tumors for immune evasion. Melanomas are transcriptionally privileged to produce energy by high-rate glycolysis resulting in tumor acidification. This tumor acidosis induced a cAMP-mediated expression of the transcriptional repressor ICER in melanoma-infiltrating macrophages of tumor-associated macrophages (TAM), leading to functional polarization of TAM towards a non-inflammatory M2 phenotype promoting tumor growth. In contrast to that, B16 F10 is an adipocytokine-producing tumor that leads to a non-inflammatory immune response, highlighting the complexity of tumor-immune interactions.

We identified highly immunogenic peptide epitopes for the development of cancer vaccination strategies. The evidence that priming of tumour specific CD8+ T cells relies on cross-presentation of tumour debris by dendritic cells (DCs) is compelling. However, it remains unclear whether the cellular localisation of mutated cancer proteins plays a role in the ability of CD8+ T cells to be primed by cancer cells. To address this question, we have analysed the ability of T-cells to recognise and lyse HLA-A*02:01-positive AML with mutated NPM1 derived peptides are presented on AML and that CLAVEEVSL is a neoantigen that can be efficiently targeted on AML with mutated NPM1 gene. Interestingly, co-infusion of TCR-transduced CD8 and CD4 T-cells resulted in superior anti-tumor reactivity compared to TCR-transduced CD8 T-cells alone. This data show that mutated NPM1-derived peptides are presented on AML and that CLAVEELSD is a neoantigen that can be efficiently targeted on AML with mutated NPM1 gene. TCR gene transfer in a co-receptor independent fashion. Immunotherapy targeting mutated NPM1 may therefore contribute to treatment of AML. This research was supported by the Dutch Cancer Society.

WS.B1.06.06
Enhanced immunogenicity of mitochondrial localised mutated proteins in cancer cells

G. Prota, L. Giudicelli, M. Reit, A. Luchea Viecco, J. Chen, J. Rehwinkel, A. Ahmed, J. Enrriquez, V. Cerundolo; 1MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Oxford, United Kingdom, 2Centro Nacional de Investigaciones Cardiovasculares Carlos III (F.S.P.), Madrid, Spain, 3Huffield Department of Obstetrics and Gynaecology, University of Oxford, UK, Oxford, United Kingdom.

The evidence that priming of tumour specific CD8+ T cells relies on cross-presentation of tumour debris by dendritic cells (DCs) is compelling; however, it remains unclear whether the cellular localisation of mutated cancer proteins plays a role in the ability of CD8+ T cells to be primed by cancer cells. To address this question, we have analysed the ability of the full length NY-ESO-1 protein and ovalbumin localised either in mitochondria or cytosol to elicit in vivo antigen specific CD8+ T-cell responses and compared direct vs cross-presentation. Our results show that cytoplasmic proteins are rapidly degraded and elicit strong direct presentation in vitro of processed epitopes. In contrast, mitochondrial localised proteins are significantly longer lived, but are downregulated from proteasomal degradation and downregulated, and are capable of eliciting in vivo significantly stronger antigen specific epitope T-cell responses than cytosolic proteins. We showed that such enhanced response of mitochondrial localised proteins is mediated by cross-presentation events dependent on STING activation and by uptake of mitochondria by CD103+ and CD11b+ DC. Finally, we have extended these findings in humans by isolating several CD8+ T- clones specific to defined epitopes expressed by mitochondrial localised proteins from one endometrial cancer patient with high mutational burden due to loss of function of the proof reading polymerase POLE. In conclusion, these findings demonstrate the greater immunogenicity of mitochondrial localised mutated proteins, highlighting strategies to identify highly immunogenic peptide epitopes for the development of cancer vaccination strategies.

WS.B2.01.01
Environmental regulation of anti-tumor responses

WS.B2.01.01.01
High glycolytic tumors evade immune destruction by acidiostasis-dependent induction of ICER in tumor-associated macrophages

T. Bohn, S. Rapp, M. Kleini, S. Pektor, K. Renner, M. Kreutz, V. Rappi, K. Gerlach, B. Wengmann, C. Luckel, M. Huber, C. Becker, E. von Stebut-Borschitz, H. Schöll, E. Schmitt, T. Bopp; 1Institute for Immunology, Mainz, Germany, 2Institute for Preventive Cardiology, Mainz, Germany, 3Institute for Nuclear Medicine, Mainz, Germany, 4Internal Medicine III, Regensburg, Germany, 5Department of Medicine 1, Erlangen, Germany, 6Institute for Medical Microbiology and Hospital Hygiene, Marburg, Germany, 7Dermatology, Mainz, Germany, 8Dermatology, Köln, Germany.

Aggressive types of skin cancer have become more common over the last 25 years. Immune checkpoint inhibitors have revolutionized melanoma treatment. However, only less than 40% of patients benefit from this therapy suggesting additional immune evasion mechanisms. To develop new innovative therapeutic strategies, detailed understanding of tumor- and micro-environmental mechanisms contributing to inefficient anti-tumor immune responses is essential. Therefore, we focused our work on identifying signaling pathways and molecules involved in melanoma formation and anti-tumor immune responses.

We explored a molecular mechanism of non-classical physicochemical communication deployed by high glycolytic tumors for immune evasion. Melanomas are transcriptionally privileged to produce energy by high-rate glycolysis resulting in tumor acidification. This tumor acidosis induced a cAMP-mediated expression of the transcriptional repressor ICER in tumor-associated macrophages (TAM), leading to functional polarization of TAM towards a non-inflammatory M2 phenotype promoting tumor growth. In contrast to that, B16 melanoma-infiltrating macrophages of ICER-deficient mice possess an inflammatory anti-tumor M1 phenotype which results in a spontaneous rejection of high glycolytic tumors by ICER-deficient mice, as well as mice with a conditional ICER in macrophages.

To test the “druggability” of CD3exCD19 modulation in melanoma treatment we conducted in vivo experiments with therapeutic application of the Adenylate cyclase inhibitor MDL-12. Inhibition of de novo cAMP synthesis lead to a rejection of B16 melanomas in C57Bl/6J mice. Taken together, our findings indicate an evolutionarily conserved mechanism of physicochemical communication between non-lymphoid tissue and the immune system that is exploited by high glycolytic tumors for immune evasion.
WS.B2.01.02
Carcinoma-associated pancreatic stellate cells induce CD4+ and CD8+ T-cell exhaustion

L. Gorchi1, C. Fernández More2, G. Mengi1, E. Randello1, H. Kaper1

1Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, 2Department of Pathology/Cytology, Karolinska University Hospital, Stockholm, Sweden.

Carcinoma-associated pancreatic stellate cells (CAPSCs) are the major type of stromal cells in pancreatic ductal adenocarcinomas and besides their pathological release of extracellular matrix proteins they are also perceived as key contributors to immune evasion. Despite the known relevance of tumour infiltrating lymphocytes in cancers, the interactions between T-cells and CAPSCs remain largely unexplored. Here, we found that CAPSCs isolated from tumors of pancreatic cancer patients expressed higher levels of the PD-1 ligands PD-L1 and PD-L2 compared to primary skin fibroblasts from healthy donors. CAPSC strongly inhibited T-cell proliferation via both contact-dependent and independent mechanisms. Blocking the activity of prostaglandin E2 (PGE2) partially restored the proliferative capacity of both CD4+ and CD8+ T-cells. After stimulation, the proportion of proliferating T-cells expressing HLA-DR and the proportion of memory T-cells was decreased when CAPSCs were present compared to T-cells proliferating in the absence of CAPSCs. Interestingly, proliferating T-cells had a greater expression of the immunosuppression markers TIM-3, PDL-1, CTLA-4 and Lag-3 in the presence of CAPSCs. Functional assays showed that T-cells expressing immune checkpoints produced less IFN-γ, TNF-α, and CD107a after restimulation when CAPSCs had been present. Thus, this indicates that CAPSCs induce expression of immune checkpoints on CD4+ and CD8+ T-cells, which contribute to a diminished immune function. This study was funded by the Swedish Cancer Society (Drn 2017:748) and the Cancer Society in Stockholm (Dnr 1711/03).

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The tumour microenvironment supports the self-renewal of tumour-promoting macrophages in colon adenoma

I. Songi1, J. Sheng, Q. Chen, K. Karjalainen, C. Ruedi1

Nanyang Technological University, Singapore, Singapore.

Circulating CCR2+ monocytes are crucial in maintaining the adult tissue-resistant F4/80+MHCII+ macrophage pool in the intestinal lamina propria. Here, we delineated murine tissue-resident F4/80+ macrophages and identified a CCR2-independent F4/80+MHCII+ macrophage subset as the only F4/80+ subset present in the embryonic lamina propria that was gradually lost after birth and almost entirely replaced by CCR2-dependent F4/80+MHCII+ macrophages in the adulthood. However, in primary human tissue, we identified a CCR2-independent F4/80+MHCII+ macrophage pool that was not only preserved in our patient cohort but also became the dominant tumour-associated macrophages within large tumours. Furthermore, by utilizing K14-CreERT2;R26-GFP fate-mapping mouse model we demonstrated that in the tumour microenvironment both macrophage fractions were able to maintain their numbers mostly independent of bone marrow contribution. Instead, they upregulated the expression of numerous genes related with cell proliferation, which may represent the driving source of immune suppression. Analyses of colon adenomas revealed that CCR2+ could be a key facilitator of macrophage self-renewal. Thus, as the intestinal environment switches from healthy to tumoural there is a corresponding shift in the renewal of tissue-resident macrophages, from bone-marrow dependent to self-maintenance through in situ proliferation, conferred by the local niche.

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WS.B2.02 Tumor immune surveillance and evasion

WS.B2.02.01 Tumor immune surveillance arises through loss of TNF sensitivity

J. Oliaro, C. Kearney, S. Vervoort, R. Johnstone;
Peter MacCallum Cancer Centre, Melbourne, Australia.

Immunotherapy has revolutionized outcomes for cancer patients, but the mechanisms of resistance remain poorly defined. Here, we used a series of whole genome CRISPR-based screens performed in vitro and in vivo to identify mechanisms of tumor immune evasion from cytokotic lymphocytes (CD8+ T cells and natural killer (NK) cells). Deletion of key genes within the TNF signaling, IFN-γ signaling, and antigen presentation pathways provided protection of tumor cells from CD8+ T cell-mediated killing, and blunted anti-tumor immune responses in vivo. In addition, a number of genes in the TNF pathway also emerged as the key mechanism of immune evasion from primary NK cells. Remarkably, we found that tumors delete the same genes when exposed to perforin deficient CD8+ T cells, demonstrating that the dominant immune evasion strategy utilized by tumor cells is acquired resistance. In cell-derived cytokotic mediated anti-tumor effector killing is a potent T cell effector mechanism capable of killing antigen-negative tumor cells. In addition to highlighting the importance of TNF in CD8+ T cell mediated killing of tumor cells, our study also provides a comprehensive picture of the roles of the TNF, IFN and antigen presentation pathways in immune-mediated tumor surveillance.

WS.B2.02.02 IL-33/ST2 signaling promotes the survival and proliferation of AML/Eto leukemic stem cells

P. Nüff, R. Rapoport1, C. Rietkerk1, A. F. Ochsenbein1,2;1Department for BioMedical Research, Bern, Switzerland, 2Department of Medical Oncology, Bern, Switzerland.

Interleukin (IL)-33 is an interleukin released upon cell necrosis and binds to the heterodimeric receptor ST2/IL1RAP, which is expressed on a subset of immune and epithelial cells. BCR/ABL transformation in chronic myeloid leukemia (CML) stem cells was shown to induce ST2 expression and propel malignant cell growth; however, the role of IL-33/ST2 signaling in acute myeloid leukemia (AML) is currently unknown. Gene expression analysis of human blood and CD34+CD38+ AML samples harboring variety of translocations revealed that ST2 was particularly upregulated on AML/ETO transformed AML cells, but not expressed on other AML subtypes and healthy hematopoietic stem cells (HSCs). We next generated an in vivo AML model by transfecting murine hematopoietic stem cells with an AML/Eto lentiviral construct. AML/ETO transformed leukemic stem cells (LSCs) highly expressed ST2 and showed increased colony formation in recombiant IL-33 (hIL-33) conditioned methylcellulose-based culture assays when compared to controls. Interestingly, amongst all screened human AML/ETO LSCs a heterogeneity in ST2 expression was observed. Molecular profiling of the LSCs revealed a negative correlation of ST2 gene expression and key genes of the Notch pathway. Blocking the Notch pathway in the ST2-AML/ETO LSCs using siRNA revealed that ST2 protein expression could be restored. Furthermore, siRNA induced gain of ST2 expression also restored in vitro colony formation capacity in the presence of IL-33. Taken together, these data provide evidence that IL-33/ST2 signaling plays a disease promoting role in AML/ETO transformed AML LSCs. Further investigations will focus on detailed signaling mechanisms and propose new therapeutic strategies to treat AML.

WS.B2.02.03 Characterization of CC-chemokine Receptor 6 (CCR6) and CC-chemokine Ligand 20 (CCL20) mediated immunosurveillance in the initiation and progression of malignant melanoma

D. Martin-Garcia, A. H. Eirik, A. S. Lonsdorf; Department of Dermatology, Heidelberg, Germany.

Chemokine ligand 20 (CCL20) expressed in the epidermis is a potent impetus for the recruitment of subsets of DCs, B-cells and memory T-cells expressing chemokine receptor 6 (CCR6), its exclusive receptor. CCL20 and a corresponding CCR6-expressing immune cell infiltrate have been detected in several malignancies, including melanoma. Yet, the functional contribution of the CCR6/CCL20 axis for the immune control of melanoma remains controversial. The characterization of CCR6-guided immune cell subsets and their functional contribution for the immune control of melanoma comprises the focus of this project. We evaluated the homeostatic and inducible secretion of CCL20 by different murine and human melanoma cutaneous cell lines by ELISA. Both murine (B16, Ret) and human (A375, C32) melanoma cell lines are capable of secreting CCL20 upon stimulation with pre-inflammatory cytokines in vitro. In order to determine the functional relevance of CCR6 on local tumor growth, B16 melanoma cells retrovirally transduced with a vector that constantly overexpresses CCL20 (B16-CCL20) were injected subcutaneously in C57BL/6 wt mice and congenic CCR6-knockout (CCR6ko) mice. While animals in both groups developed local tumors, we observed a significantly reduced tumor growth in CCR6ko mice. By contrast, wt and CCR6ko control groups did not display differences in tumor growth rate. Our results suggest that CCL20 interactions in the microenvironment of cutaneous melanoma may be an essential factor for local tumor growth. Preliminary experiments have pointed out a possible autocrine pathway that would affect only B16 tumor growth in CCR6ko mice, although the precise mechanisms are still being investigated.

WS.B2.02.04 Unravelling the mechanism behind aberrant CD37 expression in human B cell lymphoma

S. Eljfrink1, C. de Wilde1, M. van den Brand1, E. Jansen1, F. Doubрова-Siimer2, S. van Deventer1, C. Hess1, W. Stevens1, D. van Sprosen1, H. van Krieken2, C. Fidjela3, B. Scheijen1, A. van Spijlen;1Department of Tumor Immunology, Radboud Institute of Medical Life Sciences, Radboudumc, Nijmegen, Netherlands, 2Department of Pathology, Radboudumc, Nijmegen, Netherlands, 3Department of Hematology, Radboudumc, Nijmegen, Netherlands.

Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma and remains a clinical problem. We and others have recently shown loss of immune cell-specific tetraspanin CD37 protein in half of the DLBCL tumors, which is directly correlated with clinical outcome. Tetraspanins are membrane proteins that control the cell surface organization by forming tetraspan enriched microdomains. Hereby they are involved in cell migration, proliferation and survival, and play a role in cancer cell migration and cellular interactions. We discovered that CD37 controls signal transduction pathways that regulate the survival of B cells, and CD37-deficient mice spontaneously develop lymphomas. Genomic mutation analysis of the coding sequence of the human CD37 gene revealed a pathogenic mutation in 3/16 DLBCL tumors, resulting in aberrant expression of the protein. Other potential mechanisms of loss of CD37 expression may include alterations in the CD37 promoter region or increased DNA methylation. Mutation analysis of the promoter region of CD37-negative tumors (n=12) did not reveal any pathogenic mutations. Mspsil digestion on genomic DNA indicates increased methylation of the CD37 promoter region in CD37-negative B cell lines compared to CD37-positive B cell lines. Preliminary results from bisulfite sequencing of the promoter region and the first intron of CD37 show differential methylation in CD37-expressing cells versus CD37-negative cells. Taken together, the B-cell tetraspanin CD37 is a novel tumor suppressor that protects against malignant transformation of B cells. This work provides important insight into the microenvironment of cutaneous melanoma may be an essential factor for local tumor growth. Preliminary experiments have pointed out a possible autocrine pathway that would affect only B16 tumor growth in CCR6ko mice, although the precise mechanisms are still being investigated.

WS.B2.02.05 A generic, cost-efficient high performance capillary liquid chromatography-high resolution mass spectrometry method for quality control of peptide pools

G. Bosc-Bierne1, V. Armuzza1, C. Riether1,2, O. J. Kreuzer1;1Department of Biomedical Research, Bern, Switzerland, 2Department of Medical Oncology, Bern, Switzerland.

Synthetic peptide pools are used in antigen-specific T-cell assays, which are an important part in vaccine and immunotherapeutic clinical trials. As the analytical characterization is challenging due to the similarity of the single peptides or is expensive due to isotopic labeled standards, usually only a pre-characterization of the single peptides is performed. However, a regular quality control of the peptide mix would be highly desirable. Therefore, a cost-efficient high performance liquid chromatography high resolution mass spectrometry (HPLC-HRMS) method for quality control of a model peptide pool is developed. Peptides were synthesized using peptides&elephants proprietary libraries of individual peptides (LIPS) technology and purified by reversed-phase chromatography >90% each. The lophosphated single peptides were combined to a model peptide pool and analyzed by reversed-phase high-performance capillary liquid chromatography coupled to an orbitrap mass spectrometer. The experiment was performed in a high performance capillary column (10 cm x 0.3 mm, 3.5 µm CoreSil 3D, 100 Å) with a linear gradient of acetonitrile + 0.05% trifluoroacetic acid. Absolute quantification was accomplished based on ultraviolet spectroscopy. Identification was obtained by high resolution mass spectrometry. After optimizing the injection mode, the gradient elution, the temperature, the additives and sample preparation a model peptide pool was separated. The extracted ion chromatogram (XIC) was studied to confirm the exact masses as well as the total ion chromatogram (TIC) to identify possible degradation products. Relative quantification of capillary HPLC-HRMS showed that the method is suitable for the separation and quantitation of complex synthetic peptide pools.

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The macrophage tetraspan MS4A4A interacts with Dectin-1 and is required for macrophage-NK cell cross-talk and resistance to metastasis
A. Miatello1,2, F. Tomay1, M. De Pizzol1,2, B. Savino1, I. Melero1,3, P. Berraondo4, M. López-Botet2, A. Muntassir5, 1Department of Microbiology and Infection Immunology, Charité - Universitätsmedizin Berlin, Germany, 2Humanitas Clinical and Research Center, Pieve Emanuele, Italy, 3Graduate Program in Areas of Basic and Applied Biology (GABBA), Instituto de Ciencias Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal, 4Singapore Immunology Network (SIgN), Agency for Science, Technology & Research (A-STAR), Singapore, Singapore, 5Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Endocrinology Unit, Department of Clinical Sciences and Community Health, University of Milan, Milano, Italy, 1Humanitas University, Pieve Emanuele, Italy, 3The William Harvey Research Institute, Queen Mary University of London, London, United Kingdom.

Introduction: MS4A4A is a tetraspan-like molecule that is induced during monocyte-to-macrophage differentiation and is upregulated by M2 or M2-like signals, including IL-4, dexamethasone and tumor cell supernatants. We observed that MS4A4A is expressed by human tissue macrophages and tumor-associated macrophages in human cancers and is induced in monocyes from patients treated with methylprednisolone. However, MS4A4A function is totally unknown. Materials: Associating molecules of MS4A4A were identified by a split-ubiquitin assay and then confirmed by FLIM-FRET, co-immunoprecipitation and immunofluorescence. Conditional knock-out mice for MS4A4A (Ms4a4a−/−) and Ly40−/− were generated and MS4A4A contribution to macrophage functions was assessed both in vitro and in vivo models of cancer. Results: MS4A4A associates with the β3 integrin (αvβ3) in the heteromeric αvβ3-CD11c- and the heterodimeric αLβ2 integrins, and recruits CD11c to lipid rafts. MS4A4A is essential for the full activation of the Syk pathway downstream Dectin-1. The inhibition of Syk phosphorylation correlates with the reduced production of cytokines and reactive oxygen species by MS4A4A- lacking macrophages. MS4A4A deficiency in macrophages has no influence on primary tumor growth, but impacts on Dectin-1-driven NK-mediated resistance to metastasis. Indeed, the absence of MS4A4A on macrophages impairs NK cell recognition of antibody-coated tumor targets leading to the uncontrolled spread of Dectin-1 controlled metastatic events. Conclusion: We showed that MS4A4A interacts with Dectin-1 and is essential for innate responses driven by Dectin-1, including NK cell-mediated resistance to metastasis. Therefore, we demonstrated for the first time that Dectin-1 is an associating molecule of MS4A4A and that MS4A4A plays a key role in the regulation of macrophage activation.

BS.B2.03.02
Heparanase is required for dendritic cell-natural killer cell crosstalk and subsequent natural killer cell activation
A. Mayfosh, N. Baschuk; 1Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, Melbourne, Australia.

Heparanase is a beta-D-endoglucuronidase that cleaves heparan sulfate, a major component of the extracellular matrix and basement membrane. Heparanase expression and function have been well characterized in some leukemoid and melanoma cell lines, but its expression has only been described in natural killer (NK) cells. Here we investigate the importance of heparanase in NK cell activation and function, and the impact on tumour clearance. We first observed that heparanase-knock out (Hps-/-) mice implanted with E0717 LMB tumour cells presented more metastatic lesions in the lungs compared to WT animals, suggesting an impairment in tumour clearance. Following challenge in vivo with the viral RNA mimetic Poly(I:C), NK cells isolated from Hps-/- mice displayed reduced expression of the NK cell activation markers CD69, NKGD2, CD11b and G027, reduced interferon γ production and impaired cytotoxicity against ED771 LMB cells in vitro. Interestingly, direct cytokine stimulation of Hps-/- NK cells in vitro was sufficient to induce NK cell activation equal to that of WT NK cells. Given that Poly(I:C) signals through DCs to stimulate NK cells in vivo, we assessed the ability of Hps-/- DCs to activate WT NK cells in vitro. Indeed, Poly(I:C)- activated Hps-/- DCs were unable to induce NK cell activation equal to that of WT DCs, suggesting heparanase plays an essential role in enhancing its anti-NK cell activity function. Overall, our data reveals HPS3 as a co-stimulatory receptor capable of enhancing the therapeutic benefit of antineoplastic immunotherapies in vivo.

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CD137 (4-1BB) costimulation counteracts TGF-β1 inhibition of antibody-dependent NK cell responses against HER2+ transformed cells
M. Cabo1, R. Lozano-Rodríguez2, M. Atayó1, M. Costa-García1, S. Santana2, M. C. Ochoa3, P. Berraondo4, I. Melero1,5, M. López-Botet2, A. Muntassir5, 1Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain, 2University Pompeu Fabra (UPF), Barcelona, Spain, 3Centro de Investigación Médica Aplicada (CIMA), Pamplona, Spain, 4Clinica Universitaria de Navarra, Pamplona, Spain, 5Humanitas University, Pieve Emanuele, Italy, 1Humanitas University, Pieve Emanuele, Italy, 2Humanitas University, Pieve Emanuele, Italy, 3Centro de Investigación Médica Aplicada (CIMA), Pamplona, Spain, 4Clinica Universitaria de Navarra, Pamplona, Spain.

CD137 (4-1BB) costimulation counteracts TGF-β1 inhibition of antibody-dependent NK cell responses against HER2+ transformed cells

CD137 (4-1BB) plays a key role in the regulation of cell activation, proliferation, and survival in T cells, whereas its role in NK cells is less defined. A recent study identified CD137 as a costimulatory receptor capable of overcoming TGF-β1 immunosuppression, and provide the rationale for combinatorial strategies including CD137 agonists for NK cell recognition of antibody-coated tumor targets through CD16A triggers antibody-dependent cellular cytotoxicity and the release of IFN-γ and TNF-α. Several observations highlighted the potential contribution of CD137 to NK cell biology, including its upregulation in CD16A−/− NK cells in vivo, and its enhancement in CD16A−/− NK cells after induction of IFN-γ or IL-2. We have previously shown that CD137 interacts with the β2 integrin (αLβ2) in the heteromeric αLβ2-CD11c- and the heterodimeric αVβ3 integrins, and recruits CD11c to lipid rafts. CD137 is essential for the full activation of the Syk pathway downstream Dectin-1. The inhibition of Syk phosphorylation correlates with the reduced production of cytokines and reactive oxygen species by CD137−/− NK cells. Given that Poly(I:C) signals through DCs to stimulate NK cells in vivo, we assessed the ability of these DCs to activate WT NK cells in vitro. Indeed, Poly(I:C)- activated WT DCs were unable to induce NK cell activation equal to that of WT DCs, suggesting CD137 plays an essential role in enhancing its anti-NK cell activity function. Overall, our data reveals CD137 as a co-stimulatory receptor capable of enhancing the therapeutic benefit of antineoplastic immunotherapies in vivo.

BS.B2.03.04
B7-H6, a ligand for Nkp30, is regulated by BRD4 in acute myeloid leukemia cells
A. Baragáno Raneros, P. Díaz Bulnes, R. M. Rodríguez, B. Suárez Álvarez, C. López Larrea; 1Traslational Immunology Laboratory, Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain.

B7-H6, a ligand for NKp30, is regulated by BRD4 in acute myeloid leukemia cells

B7-H6 (B7-HEX4) is a ligand for the activating receptor NKp30 on natural killer (NK) cells and acts as a counter-receptor to CD96, a receptor whose ligand is GzmB, a perforin-related cell death mediator. B7-H6 is upregulated in a large variety of cancer types, and its expression is regulated by BRD4, a member of the BET family of proteins (BETs) that plays a role in transcriptional regulation. B7-H6 expression in AML cells is regulated by BRD4 protein and treatment with BETs inhibitors could damage the immune recognition mediated by NK cells allowing the tumor progression.
WORKSHOPS

WS.B3.03.05

Integrated identification and functional properties of mature and immature neutrophils in homeostasis, inflammation and cancer


1Imperial College London, London, United Kingdom, 2Cancer Research UK Beatson Institute, Glasgow, United Kingdom, 3University of Glasgow, Glasgow, United Kingdom.

Neutrophils are a critical component of the innate immune response and are capable of identifying and destroying pathogens as a result of their receptor expression and effector mechanisms. During cancer, severe infection and chronic inflammation increased numbers of neutrophils are produced and released from the bone marrow into the peripheral circulation, many of which are postulated to be developmentally immature. Neutrophil maturation within the bone marrow is essential to their development of distinct granules that are crucial to neutrophil function in host defence. We have identified and developed methods that efficiently distinguish immature from mature neutrophils in vitro and in vivo that can be utilised for their direct study.

Neutrophils are thought to worsen pathology in cancer and severe infection by both direct mediator production and suppression of other leukocytes. We identified immature Ly6G+Ly6C+ and mature Ly6GLy6C neutrophils by flow cytometry, transgenic reporters and adoptive transfer experiments. We studied localisation and function of these immature populations in colony stimulating factor 3 (CSF3; G-CSF) treated mice, lipopolysaccharide treated mice, and multiple genetically-engineered mouse cancer models. In these models, neutrophil populations alter their cell surface adhesion and chemokine receptor expression, compared with mature neutrophils. Interestingly, immature neutrophils also differ in their capacity to generate reactive oxygen species production with and without stimulation. We also measured the differential capacity of these populations to suppress cytotoxic lymphocyte activity. Overall, circulating immature neutrophils are present in models of cancer, severe infection and emergency granulopoiesis where they display different functional capacity compared to mature neutrophils.

WS.B3.03.06

Epithelial damage and tissue y6 T cells promote a unique tumour-protective IgE response

G. Crawford1, R. Castro Seane12, S. Ward1, M. Hayes1, M. Hanif1, D. Dunn-Walters1, J. Strid1

1Molecular medicine, Aachen, Germany, 2Department of Microbiology and Immunology, Melbourne, Australia.

IgE is an ancient and conserved immunoglobulin isotype with potent immune function. Nevertheless, the regulation of IgE responses remains enigmatic and evidence for a role of IgE in host defense is limited. We have previously described that IgE is strongly induced through the skin as part of the lymphoid stress-surveillance (LSS) response. LSS refers to the capacity of resident cutaneous γδ TCR+ intraepithelial lymphocytes (IEL) to directly sense epithelial cell dysregulation and initiate a restorative response. Here we explore the effector pathways leading to IgE following skin exposure to environmental xenobiotics and the role of this endogenous IgE in controlling spontaneous tumour growth. We demonstrate the development of a potent auto-reactive IgE response following topical exposure to the common environmental xenobiotic and carcinogen, 7,12-Dimethylbenz(a)anthracene. Further we show how tissue γδ TCR+ IELs are uniquely initiating and regulating the IgE, but not IgG1, response. High-throughput antibody sequencing reveals that y6 T cells shape the IgE repertoire by supporting specific VDJ rearrangements with unique CDR3 characteristics. This endogenous IgE response, via the FeCRI, protects against epithelial skin carcinogenesis and FeCr1a expression in human squamous cell carcinoma correlates inversely with poor disease outcome. Thus, LSS promotes a unique IgE response, which is part of an early host defense mechanism providing protection against cancer. Our data provides experimental support for the ‘toxin hypothesis’, suggesting IgE as an intentional host defense mechanism against non-infectious cancer-damaging environmental xenobiotics and propose a cooperative role for T and B cell immune surveillance in epithelial tissues.

WS.B3.01 Molecular regulation of T cell responses

WS.B3.01.01

Resident CD4+ T cells in secondary lymphoid organs

S. Roy1

1Department of Microbiology and Immunology, Melbourne, Australia.

Adaptive immunity relies on controlled lymphocyte migration and proper lymphocytes positioning. We and others established in vivo cell tracking based on photoconvertable proteins. Photoconverters enable the labelling of immune cells in situ and is thus particularly suited for studying cell egress and migration. To enable long-term tracking of photoconvertable cells, we established a histone-fused photoconvertable Dendra2 protein. Retroviral overexpression of histone-fused Dendra2 and in vivo cell tracking allowed us to identify a population of lymphoid node (LN) resident CD4+ T cells. In this project we analyse the molecular and functional characteristics of LN resident CD4+ T cells. We use a novel transgenic mouse with stable expression of histone-fused Dendra2 in immune cells. Naïve CD4+ T cells typically leave LNs after 8-12 hours. Recently activated T effector cells stay in LNs for approximately 3 days. We have identified another migratory pattern: resident CD4+ T cells remain in peripheral LNs for at least up to 28 days. Resident CD4+ T cells constitute 20-50% of all effector/memory CD4+ T cells, indicating a major population of effector/memory CD4+ T cells with hitherto unappreciated migratory behaviour. Our data indicate that generation of resident CD4+ T cells requires strong immune stimulation and sustained availability of antigen. Resident CD4+ T cells are maintained in a model of induced T cell receptor deficiency. This indicates that CD4+ T cells can remain resident in LN independently of continuous T cell receptor triggering. At present, we further characterise the mechanisms to generate and maintain resident CD4+ T cells.

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Production of extracellular adenosine by CD73+ dendritic cells is crucial for induction of T cell hyporesponsiveness

C. Silva-Vilches1, K. Mahone2

1University Hospital Heidelberg, Heidelberg, Germany.

Dendritic cells (DCs) express the ecto-5’-nucleotidase CD73 that generates immunosuppressive Adenosine (A) by dephosphorylation of extracellular Adenosine-monophosphate. To investigate whether A that is produced by CD73+ DCs affects induction of tolerance in contact hypersensitivity (CHS) reactions, C57BL/6 wildtype (WT) and CD73- animals were tested in a 2,4-dinitrofluorobenzene (DNFB) induced CHS model. In this model pre-treatment with 2,4-dinitrofluorobenzene (DNFB) induces tolerance to DNFB. We demonstrate that treatment with DNFB induced tolerance to DNFB only in WT but not in CD73- mice. Analysis of DCs that migrated from skin to draining lymph nodes (sDLNs) in WT mice revealed increased expression of CD73 after application of DNFB and a lower expression of activation markers (CD80 and CD86) compared to CD73- sDLNs, accompanied by elevated concentrations of extracellular A within the LN tissue. Also, markers of T cell anergy, namely early growth response protein-2 (EGR2) and N-Myc downstream regulated protein-1 (NDRG1) were upregulated in LN T cells of DNFB-treated WT mice. Moreover, those T cells from WT animals exhibited less proliferation, less activation and less cytokines production than T cells from CD73- mice upon ex vivo re-stimulation. Similar effects were observed in vitro. I.e., A+ producing WT DCs, but not CD73- DCs rendered T cells hyperresponsive, decreased their T cell costimulatory signaling and induced upregulation of EGR2 and NDRG1. Thus, our data demonstrate that expression of CD73 by DCs, which triggers elevated levels of extracellular A, is a crucial mechanism for the induction of anergic T cells and tolerance. Funded by SFB/TR156

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EGFR-HIF1a signaling positively regulates the development of Th9 cells

S. Roy1, R. Rizi1, A. Awasthi1

1Translational Health Science and Technology Institute (THSTI), Faridabad, India.

Epidermal growth factor receptor (EGFR) is crucial for the proliferation and differentiation of immune cells and is important for the development of tumors. EGFR has been shown to be expressed on T cells and play a role in T cell differentiation and regulation. The role of EGFR and its ligands in IL-9 producing Th9 cells has not been identified yet. Th9 cells play a critical role in inducing anti-tumour immunity, allergic inflammation as well as in autoimmune. To understand the transcriptional network of Th9 cells using RNAseq analysis, we identified a strong upregulation of EGFR expression in Th9 cells. We have identified that the expression of EGFR is required for the induction of IL-9 in Th9 cells, as EGFR specific inhibitor, Gefitinib, suppressed the development of Th9 cells. Further analysis of EGFR pathways in Th9 cells revealed EGFR activation upregulates hypoxia-inducible factor 1α (HIF1a) and inducible nitric oxide synthase (iNOS) in Th9 cells. The increased expression of HIF1a and iNOS increases the production of amphiregulin, an EGFR ligand in Th9 cells, which acts in a feed-forward loop further supporting Th9 cells differentiation. Mechanistically, we have identified that HIF1a binds and transactivates iNOS and IL-9 in Th9 cells. Furthermore, inhibition of EGFR and HIF1a pathway suppressed IL-9 production in marrow derived human Th9 cells and substantially promoted the tumor development. Our findings thus identify a critical role of EGFR-HIF1a module in the development and functions of Th9 cells which can be targeted for successful immunotherapy.

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**WS.B3.01.04**

Post-transcriptional regulation of cytokine production in T cells by RNA binding proteins

B. Popovic, J. F. Sperling, S. Engel, F. van Alphen, A. Guislain, B. R. Nicolet, M. C. Wolters*

1Sanquin Research/Landsteiner Laboratory, Amsterdam, Netherlands, 2Sanquin Research, Amsterdam, Netherlands.

Effective cytokine CDB8 T cell responses depend on the concerted production of inflammatory cytokines and cytokotoxins. The co-production of IFNγ, TNFa and IL-2 is a hallmark of the most potent effector cells. We recently showed that post-transcriptional regulatory mechanisms are key to fine-tune cytokine production. Both the translational rate and stabilization of cytokine mRNA was a determinant of production levels. Intriguingly, all three cytokines used private regulatory mechanisms for their production: whereas the production of TNFa almost exclusively depended on the de novo translational rate, IL-2 primarily depended on de novo transcription, and mRNA stabilization was only used to amplify the production rate. IFNγ used all three regulatory mechanisms for optimal production, i.e. de novo transcription, translation efficiency and mRNA stabilization. Here we set out to identify which factors drive this individual regulation of cytokine mRNA, with a specific focus on regulatory RNA binding proteins (RBPs). To this end, we employed streptavidin-binding RNA aptamers containing the 3'Untranslated Region (3'UTR) of the three mRNAs to identify RBPs binding to the individual 3'UTR. Using lysates from in vitro expanded, resting primary human T cells, we identified differential binding of RBPs by mass spectrometry analyses to each individual cytokine 3'UTR. We currently investigate the mode of action of the identified RBPs to fine-tune cytokine production. Unraveling the post-transcriptional mechanisms that define the cytokine production in T cells should yield valuable biological insights that will help driving effector responses against pathogens and malignant cells.

**WS.B3.01.05**

Lysosome-related organelle tethering controls directionally distinct cytokine transport in T cells

B. Qu, Y. Zhou, R. Zhao, E. C. Schwarz, R. Akbar, V. Pattu, V. Helms, H. Rieger

Saarland University, Homburg, Germany.

Cytokines are small proteins playing a key role in orchestrating activation and responses of immune cells. It is of markedly importance to target cytokines to the desired destination. In CDB4 T cells, in particular, cytokines are delivered in two distinct modes: either exclusively to the immunological synapse (IS) (eg. IL-2) or transported multi-directionally (eg. TNFa). However, the molecular mechanisms responsible for these distinct transport patterns remain elusive. Here we show that in primary human CDB4 T cells both TNFa and IL-2 vesicles tether with lysosome-related organelles (LROs), mediated by the SNARE protein Vti1b. Only LRO-tethered but not untethered cytokine vesicles are preferentially transported to the desired secretion sites, namely LRO-tethered IL-2 to the IS and LRO-tethered TNFa multi-directionally. LRO tethering enhances the recruitment of kinesin and dynein to TNFa vesicles, favoring the transport to both directions along microtubules. In contrast, IL-2 vesicles is selectively enhanced in LRO-tethered IL-2 vesicles, promoting minus-end transport to the PMTC, which localizes at the IS. Our findings suggest that LRO tethering serves as a novel mechanism to regulate directionally distinct cytokine transport, especially in CDB4 T cells.

**WS.B3.01.06**

Targeting E3 ubiquitin ligase receptor cebrobl is required for Myc-driven metastasis in CDB8 T cells

R. Hesterberg, A. A. Akkof, M. S. Beatry, W. E. Goodheart, M. Fernandez, J. L. Cleveland, P. K. Epling-Burnette

 Moffitt Cancer Center, Tampa, United States.

Derivatives of thalidomide known as immunomodulatory drugs (IMID) bind to and elicit anti-cancer and immune modulating activity by binding to the E3 ubiquitin ligase substrate receptor cebrobl (CRBN). While IMIDs potentiate T cell effector functions in healthy and immunosuppressed cancer patients, the physiological roles of CRBN in T cells is undefined. To understand the immunological role of CRBN in T cells, we made use of a germline Crbn knockout mouse (Crbn-/-) and created OT1-Crbn-/- mice as well as LckCre,Crbn-/- mice. T cells derived from Crbn-/- mice manifest higher rates of proliferation and cytokine production and an elevated bioenergetics profile, with supraphysiological levels of polyamines resulting in enhanced endoreplication and amino acid transport and increased expression of metabolic enzymes including ornithine decarboxylase related to stabilization of c-Myc. To determine if these changes occur in the presence of IMID treatment, we examined Myc expression and metabolic rates after T cell activation and found that both IMIDs and Crbn deficiency increased and sustained the expression of the master metabolic regulator Myc. While Crbn deficient T cell can exacerbate graft-versus-host (GVHD) disease in vivo, they also endow tumor infiltrating lymphocytes with superior anti-tumor reactivity following adoptive transfer and after T cell-specific deletion of Crbn. Therefore, CRBN represents a druggable target that has a profound effect on sustaining the immunometabolism of CDB8+ T cells.

**WS.B3.02.01**

HDAC controls CDB8 T cell dependent anti-tumor immunity

C. Verindeo1,2, J. Keye1,3, F. Schmidt4, R. Siemund, R. Glauben, C. Weidinger1,2

1Charité - Universitätsmedizin Berlin, Medical Department, Division of Gastroenterology, Infectiology and Rheumatology, Campus Benjamin Franklin, Berlin, Germany, 2Friedrich Schiller University, Department of Biology, Chemistry and Pharmacy, Berlin, Germany, 3Berlin School of Integrative Oncology (BSIO) MKFZ, Berlin, Germany, 4Berlin Institute of Health (BIH), Clinician Scientist Program, Berlin, Germany.

Class II histone deacetylases (HDAC) were shown to orchestrate T cell-dependent immune responses via the epigenetic control of genes and via the post-translational modification of cytoplasmic and nuclear proteins. However, the contribution of single HDAC family members to T cell differentiation and function remain elusive. To elucidate the role of histone deacetylase 7 (HDAC7) in T cells, we assessed the immune cell composition of HDac7fl/fl-Cd4-Cre mice by mass cytometry under steady state conditions using a panel of 31 classifying-, differentiation- and activation- markers, which revealed a highly pre-activated phenotype within the CD8+ T cell population. By using Seahorse analysis and RNA sequencing, we observed that deletion of HDAC7 reduced the cellular production of CDB8 T cells, due to the transcriptional deregulation of metabolism- and apoptosis-regulating genes resulting in an impaired glycolytic capacity and apoptosis of CDB8 T cells. Furthermore, HDac7 deficient CDB8 T cells harbored impaired production of IFNγ, which could be linked to reduced activation levels of Store-operated Calcium Entry (SOC) signaling. Importantly, HDac7fl/fl-Cd4-Cre mice formed significantly bigger tumors when injected with the syngenic lymphoma cell line EG7, as HDac7fl/fl-Cd4-Cre mice harbored significantly lower numbers of tumor infiltrating CDB8+ T cells in comparison to wild type littermates. Taken together our data reveal that HDAC7 might serve as a key regulator of T cell mediated anti-tumor immunity via controlling the metabolism and calcium homeostasis of CDB8+ T cells.

**WS.B3.02.02**

Deciphering the molecular pathways underlying oncogenic IL-7R signaling in T-cell acute lymphoblastic leukemia

A. Murcia Ceballos, F. Fuentes Villarejo, S. González García, M. Garcia Petydró, J. Alcain, E. García Martinez, A. A. Fernandez, M. Toribio*

1Centro de Biología Molecular “Severo Ochoa”, Madrid, Spain, 2Institute for Cancer Genetics, New York, United States.

Introduction: Interleukin-7 plays a crucial role in normal T and B-cell development and also contributes to proliferation in acute lymphoblastic leukemia (ALL), the most common childhood cancer. Recently, IL-7R mutations have been described in 10% of B-ALL cases, and mutational activation of IL-7R was suggested to be involved in T-cell leukemogenesis. We thus sought to investigate intracellular pathways underlying oncogenic IL-7R signaling in T-ALL, with the final aim of delineating specific therapeutic strategies. Methods: We investigated the impact of a novel gain-of-function mutation located in IL7R exon6 identified in T-ALL patient, by expression in cytokine-dependent and independent cell lines, and primary human early thymic precursors and T-ALL cells. Fetal thymus organotypic cultures (FTOC) allowed us to assess the step-wise oncogenic impact of mutant IL-7R in T-cell development. Moreover, biochemical approaches were performed in combination with in vitro cell proliferation and in vivo tumor progression assays of cells expressing mutant IL-7R.

Results: We show that IL-7R promoted the selective expansion and accumulation of primary human pre-T cells in FTOC, and the constitutive proliferation and survival of cytokine-dependent cells in vitro. More importantly, it also enhanced tumor progression of cell lines and primary T-ALL cells in vivo. This functional impact was associated with constitutive JAK-STAT, MAPK and mTORC1 activation and c-myc expression, independently of PI3K-Akt activation. Possible pathways underlying mutant IL-7R-dependent constitutive mTORC1 activation will be discussed.

Conclusions: The identification of mTORC1 activation through an Akt-independent pathway, points to novel molecules as promising and unexplored therapeutic targets for T-ALL.

**WS.B3.03.04**

Deciphering the molecular pathways underlying oncogenic IL-7R signaling in T-cell acute lymphoblastic leukemia

A. Murcia Ceballos, F. Fuentes Villarejo, S. González García, M. Garcia Petydró, J. Alcain, E. García Martinez, A. A. Fernandez, M. Toribio*

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Results: We show that IL-7R promoted the selective expansion and accumulation of primary human pre-T cells in FTOC, and the constitutive proliferation and survival of cytokine-dependent cells in vitro. More importantly, it also enhanced tumor progression of cell lines and primary T-ALL cells in vivo. This functional impact was associated with constitutive JAK-STAT, MAPK and mTORC1 activation and c-myc expression, independently of PI3K-Akt activation. Possible pathways underlying mutant IL-7R-dependent constitutive mTORC1 activation will be discussed.

Conclusions: The identification of mTORC1 activation through an Akt-independent pathway, points to novel molecules as promising and unexplored therapeutic targets for T-ALL.
WS.B3.02.04 Intratinal signaling visualization reveals that transient interactions between T regulatory cells and classical DC support cancer immune tolerance

F. Marangoni1, E. Carrizza1, V. Mani1, M. Theiler1, J. Prüssmann1, F. R. Mempel1
1Massachusetts General Hospital - Harvard Medical School, Charlestown, United States, 2Uniklinik Köln, Köln, Germany.

The recognition of cognate antigen within tumors is necessary for CD4+CD25+ regulatory T cells (Treg) to enforce local immunological tolerance (Bauer ICI 2014). However, how cellular interactions, tightly controlled in space and time, regulate this process in vivo is unknown. We visualized Treg activation using an NFAT-GFP construct whose nuclear localization reports the activation of TCR-calcineurin-NFAT signaling (Marangoni Immunity 2013). NFAT-GFP-transduced Treg were transferred in mice with red-fluorescent CD11c+ cells, and imaged within implanted tumors by multiphoton microscopy. We found that ~30% of polyvalent Treg, but no conventional CD4+ T cells (Tconv), were activated in tumors.

This was due to differences in the TCR repertoire because Treg and Tconvon with an identical hemagglutinin (HA)-specific TCR were activated in HA-expressing tumors. Notably, while HA-Tconv:CD11c+ interactions were stable, HA-Treg:CD11c+ contacts were short-lived. This was not due to characteristics intrinsic to Tregs but to their capacity to progressively modulate antigen presentation by APC, because HA-Treg were capable of stable interactions early after transfer but destabilized HA-Tconv:CD11c+ interactions over time. We studied the meaning of Treg:CD11c+ interactions by inducing Treg-specific deletion of calcineurin B (Cnb), which reduced tumor-associated Treg numbers, lowered the expression of ICOS and CTLA4, and delayed tumor growth. Deletion experiments identified zbl246-dependent, active signaling in the APC activating tumor-associated Treg. We thus conducted the first imaging-based investigation of Treg activation demonstrating their short-lived productive interactions with classical DC, which may be detrimental for conventional T cell function (Marangoni Immunity 2013), conversely sufficient to maintain Treg numbers and immunosuppressive capacity in tumors.

WS.B3.02.05 The CD8α-CD2 axis controls killing of leukemic B cells by cytotoxic T lymphocytes

V. Zurli1, T. Montecchi1, G. Wimmer1, O. Acuto1,2
1University of Siena, Siena, Italy, 2University of Oxford, Oxford, United Kingdom.

Introduction: Cytotoxic T lymphocytes (CTLs) play a key role in the immune defence against cancer. To exert their vital function, CTLs polarize and focally release lytic granules at the immune synapse formed with their targets. We previously reported that leukemic B cells can resist CTL killing by establishing dysfunctional synapses characterized by non-polarized granule release. In the current work, we address the mechanisms controlling lytic granule polarization in human CTLs.

Methods: Firstly, we identified the factor responsible for the formation of dysfunctional synapses by analyzing comparative proteomics analysis of surface proteins to a panel of B cells, either susceptible or resistant to CTL lysis. Then we used CRISPR/Cas9 technology, functional assays, and phospho-proteomics to characterize molecules involved in the lytic granule movement in CTLs.

Results: We identified four co-stimulatory molecules highly expressed on susceptible B cells: CD8α, CD30, SLAMF1 and SLAMF7. Among these, only CD8α, through the interaction with its receptor CD2 on CTLs, was able to promote granule polarization towards the synapse. Notably, we found that CTL costimulation through CD2 was of crucial importance for B cell killing. We are currently applying a phospho-proteomics approach to elucidate the CD2 signaling network implicated in granule polarization.

Conclusions: Considering that loss of CD8α expression on B-cell tumors has been described as the mechanism of cancer progression, our results both highlight new key aspects of CTL biology and are expected to be valuable for the development of focused therapies counteracting cancer immune evasion.

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WS.B3.02.06 Functional anti-TIGIT antibodies modulate T cell responses in vivo

1University of California, Los Angeles, David Geffen School of Medicine, Los Angeles, United States, 2University of California, Los Angeles, David Geffen School of Medicine, Los Angeles, United States, 3Immunogen Inc., Pleasanton, United States, 4University of California, Los Angeles, David Geffen School of Medicine, Los Angeles, United States, 5University of Oxford, Oxford, United Kingdom, 6University of California, Los Angeles, David Geffen School of Medicine, Los Angeles, United States, 7University of California, Los Angeles, David Geffen School of Medicine, Los Angeles, United States, 8Mount Holyoke College, South Hadley, Massachusetts, United States.

Introduction: The selective engagement of inhibitory receptors plays an important role in maintaining immune homeostasis and their divergent expression gives rise to a broad range of pathologies such as autoimmunity, cancer and chronic infections. Blocking antibodies directed against co-inhibitory receptors such as PD-1 and CTLA-4 show great efficacy as a cornerstone drug in the clinic because of their potential to restore T cell responses in vivo. TIGIT is a novel co-inhibitory receptor that has recently gained attention as a potential regulator of T cell exhaustion in the context of cancer as well as in ameliorating autoimmunity disorders. Materials and Methods: In order to study the immune modulatory properties of TIGIT, we generated a panel of functional anti-TIGIT antibody clones using hybridoma cultures and tested them in both autoimmune and cancer models. Results: We found that the administration of the agonistic anti-TIGIT antibody ameliorates autoimmune disease severity in EAE, whereas administration of the blocking anti-TIGIT antibody in combination with anti-PD-1 blockade showed a synergic anti-tumor effect in models of colon carcinoma and glioblastoma. Conclusions: Collectively, our data demonstrates that TIGIT modulation can be used to effectively regulate T cell responses and disease outcome in vivo and provides further insight for the development of novel therapeutic approaches.

WS.B3.03.01 Loss of Nk in thymic epithelial cells leads to fatal autoimmunity through aberrant Treg development

C. Hoffmann1, F. Mai1, E. Terskikh1, M. Spalinger1, B. P. Leung1, A. Wassen1, B. Becker1
1Inst. of Experimental Immunology, University of Zürich, Zürich, Switzerland, 2University of Southern California, Department of Physiology & Biophysics, Biurk, 3Robert Bosch University, Mainz, Inst. for Molecular Medicine, Mainz, Germany.

Medullary thymic epithelial cells (mTECs) mediate central T cell tolerance through negative selection. mTECs are, however, also critical for the formation of peripheral tolerance by instructing the development of nTregs. Which of these two functions dominate is a matter of some debate. We have generated a mouse model in which the development and function of mTECs are compromised by conditional ablation of the NF-kB inducing kinase (NIK, encoded by Map3k14) restricted to the TEC compartment (mTEC-NIK). In contrast to germ-line deficient "RKO" mice, which develop largely normally and show severe autoimmunity only after birth, characterized by infiltration of effector T cells in various organs. The observed autoimmunity was clearly T cell mediated, as the survival of the mice could be significantly improved upon antibody mediated T cell deletion. However, whereas there was no obvious indication of augmented auto-reactivity through the loss of negative selection, Tregs, although emerging from the thymus, were dysfunctional as indicated by high-parametric single cell analysis of Treg markers and in vivo suppression assays. Conversely, adoptive transfer of normal Tregs prevented autoimmunity. Most strikingly, in thymic co-transplantation, Tregs primed by wildtype thymus were able to prevent autoimmune pathology by mTEC-NIK educated T cells. We thus postulate that Treg induction rather than negative selection of autoreactive T cell clones is the superior duty of mTECs in thymic T cell education. The author received a fellowship of the German Research Council (DFG).

WS.B3.03.03 T cell responses in health and disease

V. Zurli1, T. Montecchi1, G. Wimmer1, O. Acuto1,2
1University of Siena, Siena, Italy, 2University of Oxford, Oxford, United Kingdom.

Medullary thymic epithelial cells (mTECs) mediate central T cell tolerance through negative selection. mTECs are, however, also critical for the formation of peripheral tolerance by instructing the development of nTregs. Which of these two functions dominate is a matter of some debate. We have generated a mouse model in which the development and function of mTECs are compromised by conditional ablation of the NF-kB inducing kinase (NIK, encoded by Map3k14) restricted to the TEC compartment (mTEC-NIK). In contrast to germ-line deficient "RKO" mice, which develop largely normally and show severe autoimmunity only after birth, characterized by infiltration of effector T cells in various organs. The observed autoimmunity was clearly T cell mediated, as the survival of the mice could be significantly improved upon antibody mediated T cell deletion. However, whereas there was no obvious indication of augmented auto-reactivity through the loss of negative selection, Tregs, although emerging from the thymus, were dysfunctional as indicated by high-parametric single cell analysis of Treg markers and in vivo suppression assays. Conversely, adoptive transfer of normal Tregs prevented autoimmunity. Most strikingly, in thymic co-transplantation, Tregs primed by wildtype thymus were able to prevent autoimmune pathology by mTEC-NIK educated T cells. We thus postulate that Treg induction rather than negative selection of autoreactive T cell clones is the superior duty of mTECs in thymic T cell education. The author received a fellowship of the German Research Council (DFG).

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A temporally dynamic Fasp3 autoregulatory transcriptional circuit controls the effector Treg programme

D. Bending, P. Pietro Martini, A. Paduraru, C. Ducker, L. Marzagovan, M. Laviron, T. Crompton, M. Ono

1 University of Birmingham, Birmingham, United Kingdom, 2 Imperial College London, London, United Kingdom

Background: Understanding the mechanisms of cellular differentiation is challenging because differentiation is initiated by signaling pathways that drive temporally dynamic processes, which are difficult to analyse in vivo. Results: We establish a new Tool, Timer-of-cell-kinetics-and-activity (Tocky [toki]), in Time in Japanese). Tocky uses the Fluorescent Timer protein, which spontaneously shifts its emission spectrum from blue-to-red, in combination with computer algorithms to reveal the dynamics of differentiation in vivo. Using a transcriptional target of T cell receptor (TCR)-signaling, we establish N4a3-Tocky to follow downstream effects of TCR signaling. Using N4a3-Tocky, we determined the temporal sequence of events during regulatory T cell (Treg) differentiation, identified the major precursor population of Treg, and showed that persistent TCR signals leads to the initiation of Fasp3 transcription and Treg generation. Interestingly, in the periphery, N4a3-Tocky showed that self-reactive T cells, which include Treg and memory-phenotype T cells, received spontaneous infrequent TCR signals. Furthermore, using the murine model of Multiple Sclerosis, myelin-specific T cells at the site of autoimmune inflammation also showed persistent TCR signaling, which N4a3-Tocky distinguished from infrequent TCR signals in self-reactive T cells. In addition, by generating Fasp3-Tocky, we established that the Tocky approach can be applied to another gene as well. By analysing differentiating Treg using Fasp3-Tocky, we showed a progressive demethylation of the Fasp3 gene across time, demonstrating that the active demethylation process occurred in T cells with sustained Fasp3 transcription. Conclusion: Tocky is a tool for cell biologists and immunologists to address previously inaccessible questions by directly revealing dynamic processes in vivo.

Funder: Biotechnology and Biological Sciences Research Council

N4BP1 and TNIP1 control MHC-I display on neuroblastoma tumors


1 University of Leiden, Leiden, Netherlands, 2 Netherlands Cancer Institute, Amsterdam, Netherlands

Neuroblastoma is the second most common tumor in children. The cause of neuroblastoma is thought to originate from derailed development of embryonic neural crest cells and is accompanied by low MHC-I expression and suppression of the NF-κB transcription factor. Here, we addressed MHC-I gene regulation in neuroblastoma, with ultimate goal to enhance its immunogenic potential for therapeutic T-cell targeting. Using a genome-wide CRISPR screen, we identified N4BP1 and TNIP1 as inhibitory factors of NF-κB-mediated MHC-I expression in neuroblastoma. In support for a critical relevance of these NF-κB inhibiting factors, advanced stage neuroblastoma patients who express high levels of TNIP1 and N4BP1 by histology have worse overall survival. Depletion of N4BP1 or TNIP1 indeed increased NF-κB and MHC-I expression, and stimulated recognition by antigen-specific CD8+ T cells. We confirmed that TNIP1 inhibits canonical NF-κB member RelA by preventing activation of the RelA/p50 NF-κB dimer. Furthermore, we show that N4BP1 inhibits both canonical and non-canonical NF-κB through binding of deubiquitinating enzyme CEZANNIE, resulting in stabilization of TRAF3 and degradation of NF-κB-initiating kinase NIK. Thus, N4BP1/CEZANNIE or TNIP1 are candidate immunotherapy targets in neuroblastoma tumors that should lift NF-κB suppression, and thereby trigger increased peptide/MHC-I-mediated tumour-viability to enhance therapeutic T-cell targeting.

T cell-induced tumor vulnerability discovery in an iNOS-independent genomic landscape

D. Depper, D. Vredevoogd

1 The Netherlands Cancer Institute, Amsterdam, Netherlands

New clinical opportunities are needed to increase immunotherapy (IT) benefit. Whereas iNOS-pathway mutations cause IT resistance, we find that iNOS-receptor-deficient tumors remain remarkably susceptible to T cell elimination. To investigate transcriptional proteomics and genome-wide CRISPR-Cas9 screening with clinical data, we defined the iNOS-inhibitory network and uncovered a new immunotherapeutic opportunity. Activation of TRAF2 enhanced T-cell elimination of both melanoma and lung cancer cells by redirecting Tnf signaling to favor RIPK1-dependent apoptosis. TRAF2 loss greatly enhanced the therapeutic potential of pharmacological inhibition of its interaction partner cIAP1. By integrating transcriptomics, proteomics and genome-wide CRISPR-Cas9 screening with clinical data, we defined the IFNγ-dependent transcription and Treg generation. Interestingly, in the periphery, N4a3-Tocky showed that self-reactive T cells, which include Treg and memory-phenotype T cells, received spontaneous infrequent TCR signals. Furthermore, using the murine model of Multiple Sclerosis, myelin-specific T cells at the site of autoimmune inflammation also showed persistent TCR signaling, which N4a3-Tocky distinguished from infrequent TCR signals in self-reactive T cells. In addition, by generating Fasp3-Tocky, we established that the Tocky approach can be applied to another gene as well. By analysing differentiating Treg using Fasp3-Tocky, we showed a progressive demethylation of the Fasp3 gene across time, demonstrating that the active demethylation process occurred in T cells with sustained Fasp3 transcription. Conclusion: Tocky is a tool for cell biologists and immunologists to address previously inaccessible questions by directly revealing dynamic processes in vivo.

Funder: Biotechnology and Biological Sciences Research Council

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T cell adoptive therapy has the potential to treat human malignancies. Nonetheless, the poor immunogenicity of tumours and a strongly suppressive tumour microenvironment, drive the effector-Treg programme and are dependent on a Foxp3 protein-dependent autoregulatory transcriptional circuit, which simultaneously sustains Foxp3 transcription whilst repressing IL-2 expression. Persistent Foxp3 transcriptional activity controls the expression of coinhibitory molecules, including CTLA-4 and effector-Treg signature genes. Using N4BP1, we found an association between Foxp3 transcription and T cells and was even effective in a mouse model refractory to anti-CTLA-4 antibody therapy. Collectively, our study discloses the mechanisms behind Foxp3-driven T-cell regulation and establishes the Fasp3-Tocky system as a tool to investigate the mechanisms behind Treg-targeting immunotherapies.

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NKG2A blockade potentiates CD8+ T cell immunity induced by cancer vaccines


1 Leiden University Medical Center, Leiden, Netherlands, 2 Vanderbilt University, Nashville, United States, 3Innate Pharma, Marseille, France, 4University of Washington, St Louis, United States

T cell immunity induced by cancer vaccines is greatly potentiated by disruption of the NKG2A/Qua-1 axis. NKG2A blockade operated through CD8+ T cells and was even effective in a mouse model refractory to anti-CD8+ T cell immunity and Treg clearance, and critical clinical exploration of TRAF2 for cancer immunotherapy.

NKG2A blockade potentiates CD8+ T cell immunity induced by cancer vaccines


1 Leiden University Medical Center, Leiden, Netherlands, 2 Vanderbilt University, Nashville, United States, 3Innate Pharma, Marseille, France, 4University of Washington, St Louis, United States

Cancer vaccination has shown thus far limited clinical efficacy due to multiple suppressive factors in the tumour environment. We now demonstrate that the inhibitory receptor NKG2A constitutes an adaptive resistance mechanism during cancer vaccination by interaction with HLA-E on tumour cells. This immune receptor was preferentially found on tumour-infiltrating natural killer cells and CD8+ T cells, but not CD4+ T cells. Expression on CD8+ T cells was found on CD103+ tumour-infiltrating cells and only partly overlapped with other checkpoint receptors. Particularly high frequencies of NKG2A-expressing lymphocytes were detected in tumours with an immune-reactive profile and could be identified by therapeutic cancer vaccination. To examine if NKG2A represented an adaptive resistance mechanism during cancer vaccination, we blocked the receptor with a therapeutic antibody and performed genetic knockdown experiments for its ligand Qua-1, the conserved ortholog of HLA-E. In four mouse tumour models, the modest effect of therapeutic vaccines was greatly potentiated by disruption of the NKG2A/Qua-1 axis. NKG2A blockade operated through CD8+ T cells and was even effective in a mouse model refractory to anti-CD8+ T cell therapy. These findings indicate that NKG2A-blocking antibodies might improve clinical responses to therapeutic cancer vaccines.

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Molecular control of T cell activation and exhaustion

R. R. Brownlie, C. Garcia, M. Ravaud, D. Zehir, R. Zamosyta, R. J. Salmond

1 University of Leeds, Leeds, United Kingdom, 2 Imperial College London, London, United Kingdom, 3Technical University Munich, Munich, Germany

T cell adoptive therapy has the potential to treat human malignancies. Nonetheless, the poor immunogenicity of tumours and a strongly suppressive tumour microenvironment, characterized by high levels of inhibitory cytokines such as TGFβ, limit the efficacy of this approach. We have previously shown that T cells lacking inhibitory phosphate PTN22 have enhanced responses to weak antigens (1), which may be of benefit in a tumour setting. We now show that PTN22-deficient CD8+ T cells also have a substantially reduced susceptibility to TGFβ inhibition. Subsequently, tumour-reactive PTN22½ CD8+ T cells are superior in their ability to clear weakly immunogenic TGFβ-secreting tumours in vivo. Mechanistically, in the absence of PTN222, TCR-induced NFκB activation and IL-2 secretion is enhanced, which enables T cells to overcome the anti-proliferative effects of TGFβ (2). Furthermore, we present data showing that PTN22 also influences anti-tumour T cell memory.
Following activation by antigens, naïve CD8 T lymphocytes establish specific heritable gene expression programs that define the progression to long-lasting memory or to short-lived effector cells. While lineage specification in T cells is critical for protection, the impact of epigenetic silencing on T lymphocyte differentiation is still incompletely understood. Here, we show that a heterochromatin-mediated gene expression silencing by Suv39h1, a histone H3 lysine 9 methyltransferase plays a critical, evolutionarily conserved, role in heterochromatin structure and dynamics. We show that in murine CD8+ T cells activated after Listeria monocytogenes infection in vivo, Suv39h1-dependent H3K9me3 deposition controls the expression of a set of stem cell-related/memrory genes. Single-cell RNA sequencing analyses reveals that the silencing in stem/memory genes selectively affects terminally differentiated effector subsets. The results also include proportionally more CD8+ T effector cells with stem cell-related genes across different Suv39h1-defective CD8+ T cell sub-populations. In line with these observations, Suv39h1-defective CD8+ T cells show increased memory potential, including sustained survival and increased long-term re-programming capacity, as compared to Suv39h1-proficient CD8+ T cells. We conclude that Suv39h1 plays a critical role in marking chromatin to stem/memory gene expression during CD8+ effector T cell terminal differentiation. In doing so, Suv39h1/H3K9me3 would establish an epigenetic barrier on the stem/memory stem gene expression program, preventing the effector re-programming into memory cells. These results open new perspectives for the manipulation of epigenomic programming of T lymphocyte identity in the context of T cell-based immunotherapies.

TLR9 signaling mediates functional responses induced by direct ligation of ODN 2216 in CD4+CD25- effector T cells

R. K. Sharma, J. Sharma, P. Jain¹, A. Gupta¹, N. Sachdeva¹

1Post Graduate Institute of Medical Education and Research, Chandigarh, Chandigarh, India, 2Drexel University College of medicine, Philadelphia, Philadelphia, United States.

Oligo(deoxy) nucleotides (ODNs) are established TLR9 ligands; however, their functional responses in CD4+ T cells are believed to be independent of TLR9 and MyD88. We have recently demonstrated activation of TLR9 signaling after ODN 2216 ligation in CD4+ effector T (Teff) cells. However, the consequences of direct ODN ligation on the immunophenotype of CD4+ Teff cells remain to be elucidated. In view of this, we studied the functional responses in CD4+ Teff cells after stimulation with ODN 2216 and the role of TLR9 signaling in the observed changes in the effector phenotype. Firstly, TLR9 expression in CD4+ Teff cells was regulated by downstream molecules of TLR9 signaling in a feedback controlled fashion. Next, we observed a TLR9 signaling dependent increase in proliferation of CD4+ Teff cells stimulated using ODN 2216. Also, we observed increased synthesis of immunoregulatory molecules including TGF-β, CTLA-4 and IL-10, resulting in an anti-inflammatory phenotype similar to the Th3 type of regulatory T cells. These Th3 like cells were able to suppress the proliferation of untreated Teff cells. This expression of predominant immunoregulatory cytokine, TGF-β was found to be dependent on molecules involved in TLR9 signaling in these cells, with exception of MyD88. Collectively, our results demonstrate that ODN 2216 induced functional responses in CD4+ Teff cells depend upon TLR9 signaling pathway resulting in modulation of Teff cells. Our findings thus pave the way for future research to explore direct modulation of adaptive immune cells, using innate immune ligands, to withstand exaggerated inflammatory/autoimmune responses.

The T cell genome in 3D: how higher order chromatin structures regulate virus-specific T cell differentiation

B. E. Russ, M. Olthansky, Z. He², J. Pausen², S. Tómer³, J. Li¹, P. Callas, C. Murre¹, S. J. Turner³

1Biomedical Discovery Institute, Clayton, Australia, 2University of California, San Diego, San Diego, United States, 3University of Oslo, Oslo, Norway.

Infection triggers large-scale changes in the phenotype and function of virus-specific CD8+ T cells ensuring that they acquire the necessary lineage specific functions critical for immune clearance of the pathogen. While the molecular mechanisms that control these changes are becoming apparent, how they combine and contribute to regulate CD8+ T cell differentiation is still unclear. Genome wide mapping of chromatin interactions (HiC), histone PTMs (ChIP-seq) and chromatin accessibility (ATAC-seq) within immature thymocytes, naïve effector and memory virus-specific CD8+ T cells demonstrated that maturation of higher order chromatin structures occurs upon differentiation of CD8+ T cells from an immature to mature state. Interestingly, the chromatin structure within naïve CD8+ T cells, a stage to be pre-configured in a lineage-specific way, both at the level of histone PTMs and higher order chromatin contexts. This genomic pre-configuration is then associated with targeted epigenetic maturation of lineage-specific genomic elements upon T cell activation, thus implying that the outcome of CD8+ T cell differentiation is largely pre-determined. These data have implications better understanding of the molecular events, and their regulation, that occur during the generation of effective T cell responses and establishment of immunological memory.

Deeper phenotypic characterization of colorectal cancers by high-dimensional mass cytometry reveals tumor-specific immune landscapes

N. L. de Vries, S. van Ueren, T. R. Abdelaal, M. E. van Henk, R. van der Breggen, A. Farina Sarraqueta, K. C. Peeters, T. Höltt¹, B. L. Lieveleld¹, F. Koning, N. F. de Miranda³

1Leiden University Medical Center, Leiden, Netherlands, 2TEGObiosciences GmbH, Landsbut, Germany, 3Delft University of Technology, Delft, Netherlands.

Introduction: Immune checkpoint blockade has revolutionized cancer treatment. However, clinical outcomes are highly variable as only a proportion of cancer patients benefit. As such, an in-depth understanding of the immune cell populations that participate in the process of tumorigenesis is necessary. The aim of this study is to unravel local and systemic immune profiles of colorectal cancer (CRC) using high-dimensional immunophenotyping by mass cytometry.

Materials and methods: The expression of 36 immunomarkers was simultaneously assessed at the single-cell level by mass cytometry in tumor tissues, tumor-associated lymphoid nodes, adjacent normal mucosa, and peripheral blood samples from 18 CRC patients. Cytoplane and HONE (Hierarchical Stochastic Embedding) analyses were carried out to identify and visualize the immune composition in the tissues.

Results: We identified 218 phenotypically distinct immune subsets, including tumor-resident CD103+PD-1+ cytotoxic helper, and gamma delta T cells, CD4+ICOS+CD27+ T cells, and the recently described lineage-negative CD3+CD8+CD45RO+CD27+CD25+ intermediate-annate lymphoid cells (int-ILCs), all with an activated phenotype. These cells were exclusively found in tumor tissues. Unsupervised clustering of the tissues based on the composite immune profile separated mismatch-repair (MMR)-deficient from MMR-proficient CRCs, and showed strong correlations between the presence of int-ILCs and the CD103+PD-1+ and ICOS+ T cell populations in MMR-deficient CRCs.

Conclusions: High-dimensional immunophenotyping of CRCs reveals tumor-specific immune signatures and points towards a coordinated adaptive and innate immune response to CRC. Previously unappreciated immune cell populations further differentiate the two main pathways of CRC tumorigenesis, and suggests a multi-targeted exploitation of their anti-tumor pathways in a therapeutic setting.

A novel bispecific T cell-recruiting antibody with trivalent EGFR binding and monovalent CD3 binding for cancer immunotherapy


1Aarhus University, Aarhus, Denmark, ²Molecular Immunology Unit, Hospital Universitario Puerta de Hierro, Majadahonda, Spain, ³Leadarts, Madrid, Spain, 4Universidad Complutense de Madrid, Madrid, Spain, 5CIC BioGUNE, Donostia, Spain, 6École Polytechnique Federale de Lausanne, Lausanne, Switzerland, 7Hospital Universitario Puerta de Hierro, Majadahonda, Spain, 8Utrecht University, Utrecht, Netherlands.

The redirection of T cell activity using bispecific antibodies is one of the most promising cancer immunotherapy approaches currently in development, but it is limited by cytokine storm-related side-effects, as well as the pharmacokinetics and tumor-penetrating capabilities of current bispecific antibody formats. Here, we have engineered the ATTACK (Asymmetric Tumor Trimer Body for T cell activation and Cancer Killing), a novel T cell-recruiting bispecific antibody which combines three EGFR binding single-domain antibodies (YH1; clone EG1A) with a single CD3 binding single-chain variable fragment (scFv; clone OKT3) in an intermediate molecular weight package. The two specificities are oriented in opposite directions in order to simultaneously engage cancer cells and T cell effectors, and thereby promote immunological synapse formation. EGα1 ATTACK was expressed as a homogenous, nonaggregating, soluble protein by mammalian cells and demonstrated an enhanced binding to EGFR, but not CD3, when compared to the previously characterized tandem bispecific antibody which has one Egα1VHH and one O KT3 scFv per molecule. Egα1 ATTACK induced synapse formation and early signalling pathways downstream of TCR engagement at lower concentrations than the tandem VHH-scFv bispecific antibody. Furthermore, it demonstrated extremely potent, dose-dependent cytotoxicity when targeting human T cells towards EGFR-expressing cells, with an efficacy over 15-fold higher than that of the tandem VHH-scFv bispecific antibody.

These results suggest that the ATTACK is an ideal format for the development of the next generation of T cell redirecting bispecific antibodies.
Improving anti-CD137 immunotherapy against multiple myeloma through PD1 blockade

A. C. Picker;
Centre de Recherches en Cancérologie de Toulouse, Toulouse, France.

Multiple Myeloma (MM) is the second most common hematological malignancy in the world. MM is characterized by the development of malignant plasma cells within the bone marrow. Despite new therapies, there is no cure and therefore there is a critical need to find new treatments. Agonist monoclonal antibodies (mAbs) targeting the activation-induced costimulatory molecule CD137 (4-1BB) expressed by CD8+ T lymphocytes were recently shown to represent a promising agent against myeloma. In this study, we dissected the immunological mechanisms implicated in anti-CD137 therapy in order to potentiate the anti-MM activity of this agent. Using the most relevant mouse model for MM, the Vκ-MYC model, we found that anti-CD137 therapy induced CD8+ T cell activation, effector T cell expansion and anti-MM activity. However, we found that anti-CD137 mAbs was progressively accompanied with T cell dysfunctions and MM relapse. We found that anti-CD137 mAbs strongly increased the expression of immune checkpoint such as PD-1, Lag3 and Tim3 that may directly account for T cell defects and myeloma outgrowth. The loss of proliferative capacity and cytokine secretion by PD1+CD8+ T cells isolated from anti-CD137 treated mice confirmed this hypothesis. In addition, we showed that in vivo PD1 blockade increased effector CD8+ T cells expansion induced by anti-CD137 mAb. Furthermore anti-PD1 mAb increased significantly the survival of MM bearing mice treated with anti-CD137 mAb. Altogether our data reveal that anti-CD137 promote an exhaustive cell death by PD1- and that PD1 blockade could potentiate anti-CD137 treatment and represent a new therapeutic strategy against MM.

PD1 Signals are Critical for Maintenance of Functional CD8 T Cell Memory

S. Sarkar1, Y. Yuzefpolskiy1, F. Baumann2, K. Ernst-Bernhard1, M. Prilj1, P. Nigh2, S. Riddell1, M. Seden2, P. Morgan2, M. C. Jensen1, V. Kalia1;
1Seattle Children's Research Institute, Seattle, United States; 2University of Washington School of Medicine, Seattle, United States, 3Pennsylvania State University, University Park, United States; 4Fred Hutchinson Cancer Research Center, Seattle, United States.

Inhibitory signaling in dysfunctional/exhausted cytotoxic T lymphocytes through the PD-1 axis is well established in diseases with chronic antigen. While PD-1 is expressed at the highest levels during priming of both acute and exhausted infections, its role in long-lived antigen-independent T cell memory remains undefined. Paradoxical to its role as an inhibitory receptor, here we show that during priming and activation, PD-1 expression has minimal impact on the proliferation, size, polyfunctionality and effector program of T cells. Instead, our studies reveal an unexpected requirement of PD-1 in the maintenance of functional T cell memory, when it is expressed at significantly lower levels than recently activated or exhausted CD8 T cells. Loss of T cell intrinsic PD-1 signals led to a striking defect in homeostatic renewal, thus resulting in a precipitous decline and near ablation of the memory pool.

Notably, in the setting of PD-1 checkpoint blockade immunotherapy for chronic viral infection, where the exhausted CTLs regained function as expected, there was significant attrition of pre-existing functional memory cells to a previously administered vaccine. Metabolically, PD-1 signals were necessary to drive the critical switch from anabolic glycolysis to fatty acid oxidation program needed for bioenergetics of quiescent memory sustenance. These studies define PD-1 as a key metabolic regulator of protective T cell immunity, and have important clinical implications for pre-existing T cell memory to prior infections and vaccinations during PD-1 checkpoint blockade immunotherapy in cancer.

Combination therapy with anti-PD-L1 antibody and depletion of regulatory T cells during acute viral infections results in improved virus control but lethal immunopathology

M. Drabczik-Pluta, P. David1, E. Patzall1, Y. Kruuskche1, T. Werner1, N. Honke1, A. M. Westendorf1, K. S. Lang1, U. Ottmer1, G. Zeilský1;
1Institute for Virology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany; 2Institute of Microbiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany; 3Institute of Immunology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany.

Inhibitory checkpoints like PD-1/PD-L1 and regulatory T cells (Tregs) are important suppressive mechanisms preventing immunopathology by damping immune responses against acute viral infections. Recently, combination therapy blocking these inhibitory mechanisms was induced into the clinic for the treatment of tumor diseases. It was previously researched in an experimental combination therapy targeted PD-L1 and Tregs was also effective in chronic viral infection. However, whether such a therapy is efficient during an acute infection remains to be investigated. In the current study, depletion of Tregs combined with PD-L1 and Tim-3 blocking antibodies was used during acute Friend Retrovirus infection of mice, which normally develop only transient splenomegaly after infection. The combinatorial treatment resulted in a dramatic expansion of cytotoxic CD4+ and CD8+ T cells and a subsequent reduction of viral loads in infected organs. However, limited viral replication was accompanied by a rapid development of lethal gastrointestinal immunopathology. Similar results were obtained after combination therapy in influenza virus infected mice. Treated mice efficiently controlled influenza virus, nonetheless they died of immunopathology in the lung likely mediated by cytotoxic CD8+ and CD4+ T cells. Our findings demonstrate that multiple mechanisms control the T cell response against acute viral infections, even in those infections that normally cause only mild clinical symptoms. Immunopathology is the main complication in cancer patients treated with immune checkpoint blockade. Acute infections can strongly enhance the immunopathology associated with combination immunotherapy and therefore effective measures for infection prevention should be applied in cancer patient undergoing such treatments.

Plasticity of Tc17 cells is regulated by CTLA-4 via STAT1/3

A. Arrá1, H. Lingel2, B. Kuropak1, T. Fischer2, C. Freund1, M. Pierau1, M. Brunner-Weinzierl2;
1Department of Pediatrics, Otto-von-Guericke-University, Magdeburg, Germany; 2Leibniz-Institut für Molekulare Pharmakologie & Freie Universität, Berlin, Germany; 3Department of Hematology and Oncology, Otto-von-Guericke-University, Magdeburg, Germany.

Blockade of CTLA-4 on CD8 T cells is demonstrated to be of particular importance in enhancing effector functions of Tc1 cells by secretion of granulymyel and cytokines IFNy and TNFα in a role of CTLA-4 in regulating Tc17 cells, which are generally less cytotoxic in nature but are shown to exhibit strong anti-tumor activity due to their highly plastic nature to acquire Tc1 characteristics with increased persistence, is not completely understood. B16-melanoma model was used to investigate the effects of CTLA-4 on Tc17 cell mediated control of tumor growth. CTLA-4-mediated phosphorylation of targets in Tc17 cells was analyzed using PepScan screen. CHIP-qPCR was performed to determine STATs competence in binding to IL-17 promoter. Tc17 cells lacking CTLA-4 signaling displayed limited activation of STAT3, leading to compromised production of its target gene products such as IL-17, IL-23R and ROrly. Upon re-stimulation with IL-12, these cells displayed faster downregulation of Tc17 hallmarks and acquire Tc1 characteristics, which are known to correlate with tumor control. Mechanistically, in primary and re-stimulated Tc17 cells, STATs binding to IL-17 promoter was strongly augmented by CTLA-4, associated with less binding of STATs and reduced relative activation of STAT1, which is known to block STAT1 activity. Consistent with these findings, inhibition of CTLA-4-induced STAT3 activity reversed enhancement of signature Tc17 gene products, rendering Tc17 cells susceptible to conversion to Tc1-like cells with enhanced cytotoxic potential. CTLA-4 critically shapes the characteristics of Tc17 cells by regulating relative amounts of pSTAT3/1, which provides new perspectives to enhance cytotoxicity of antitumor responses.

De novo DNA methylation programming restraints T cell rejuvenation during immune checkpoint blockade therapy

St Jude Children's Research Hospital, Memphis, United States.

Immune-checkpoint blockade (ICB) mediated rejuvenation of CD8 T cell effector functions has emerged as one of the most promising frontiers for treating cancer and chronic infections. However, antigen-specific T cells that have experienced prolonged antigen exposure are often terminally differentiated, and have a limited capacity to mount an effector response during ICB treatment. Such exhaustion of effector potential is a major impediment of current T cell based immunotherapy efforts. Using in vivo mouse models of tumor and chronic viral infection, we assessed the role of de novo epigenetic programming in establishing ICB-refractory exhausted T cells. We observed that genetic deletion of the de novo DNA methyltransferase, Dnmt3a, in T cells at the effector stage of an immune response to chronic lympohytic choriomeningitis virus (LCMV) infection allowed antigen-specific T cells to retain highly functional despite expressing high levels of PD-1 and having prolonged exposure to antigen. Quite strikingly, PD-1 blockade treatment of chronically infected animals resulted in massive expansion of PD-1+ Dnmt3a-deficient antigen-specific T cells. Whole-genome methylation profiling of WT and Dnmt3a-deficient LCMV-specific CD8 T cells identified de novo DNA methylation programs that are coupled to development of ICB-nonresponsive virus and tumor-specific T cells. Building upon these findings, we have identified de novo epigenetic programs acquired in human tumor-associated PD-1hi CD8 T cells. Collectively, these data establish Dnmt3a-mediated de novo DNA methylation programming as a key regulator in establishing ICB-refractory exhausted CD8 T cells and highlights epigenetic reprogramming of T cells as a novel approach to enhance T cell-based cancer therapies.
Mitochondrial morphological and functional reprogramming following CD137 (4-1BB) co-stimulation

I. Esteve-Romero, S. Labiano, S. Garasa, E. Santamaria, A. Rouzaud, M. Emomarado, A. Azpilikueta, I. Inoges, E. Bolafos, M. Aznar, A. Sánchez-Paulete, S. Doncha, I. Mekentz, A. Tejeira\footnote{1}

\textit{1}Center for Applied Medical Research (CIMA), Pamplona, Spain, \textit{2}CIBERERD. Centro Virtual de la Investigación Biomédica en red de enfermedades hepáticas y digestivas, Madrid, Spain, \textit{3}the Instituto Investigaciones Cardiovasculares Carlos III (CINC), Madrid, Spain, \textit{4}CIBERONC. Centro Virtual de la Investigación Biomédica en red de Oncología, Madrid, Spain.

CD137 (4-1BB) is a costimulatory receptor of the TNFR family expressed by T and NK lymphocytes whose function is exploitable for cancer immunotherapy. Mitochondria regulate fundamental cellular processes, including the metabolism of T lymphocytes. Herein, we show that CD137 co-stimulation provided by agonist mAb and CD137. (4-1BB) induces mitochondria enlargement that results in enhanced mitochondrial mass and transmembrane potential in human and mouse CD8\(^+\) T cells. Such mitochondrial changes increase T-cell respiratory capacities and are critically dependent on mitochondrial fusion protein OPA-1 expression. Mass and function of mitochondria in tumor-reactive CD8\(^+\) T cells from cancer-bearing mice is invigilated by agonist anti-CD137 mAb. In fact, mitochondrial mass and function are baseline dependent in CD137-deficient tumor reactive T-cells. Furthermore, tumor rejection induced by the synergistic combination of adoptive T-cell therapy and anti-CD137 antibodies is critically dependent on OPA-1 expression in transferred CD8\(^+\) T cells. Moreover, stimulation of CD137 with anti-CD137 mAb in short-term cultures of human tumor-infiltrating lymphocytes leads to mitochondria enlargement and increased transmembrane potential. Collectively these data at a point a critical link between mitochondrial morphology, function and enhanced anti-tumor T-cell effector activity upon CD137 co-stimulation.

WS.C1.01.01 Regulation in tissue specific autoimmunity 1

HC.Wilson, C. Le Coz, S. Mayr, S. Marchal, J. Strohf, B. Reiniinger, T. Hammes, S. Saluzzo, P. Kalits, W. Rabitsch, G. Hopfinger, G. Story\footnote{1}

\textit{1}Department of Dermatology, Medical University of Vienna, Vienna, Austria, \textit{2}Department of Internal Medicine I, Bone Marrow Transplantation Unit, Medical University of Vienna, Vienna, Austria.

Myeloidactivating and subsequent allogeneic hematopoietic stem cell transplantation (HSCT) present unique conditions in the human system to study longevity, residency and repopulation capacities of tissue. Therefore, we profiled immune cell dynamics in 45 patients receiving HSCT as treatment for haematological malignancies. Since HSCT and general blood were taken at different time points (before start of treatment, day of transplantation, 2-14/52 weeks after HSCT) and analyzed using flow cytometry, tissue immunofluorescence and low-input RNA-sequencing of purified cell subsets. Additionally, skin sections of patients receiving sex-mismatched donor cells were assessed for X/Y-chimerism by fluorescence-in-situ-hybridization up to 8 years post transplantation.

Upon myeloidactivating, recirculating immune cells were eliminated in skin and peripheral blood and resurfaced 2-14 weeks after transplantation of donor cells. Strikingly, epidermal and dermal aT cells expressing residency markers remained stable throughout all time points analyzed. This skin-resident subset was largely CD4\(^+\), displayed proliferative potential after TCR stimulation and was competent of cytokine production. Furthermore, skin resident T cells of the recipient constituted 35\% of T cells at time of full immunological recovery (14 weeks post-transplant) and contained donor T cells up to 8 years after engraftment. Our results combine a unique clinical setting with in-depth cell profiling using RNA-sequencing and imaging techniques, painting a detailed picture of skin-resident cells as a model for T cell turnover in peripheral organs. Thus, we were able to identify a remarkably resistant and long-lived T cell population with implications for numerous inflammatory conditions including graft-versus-host-react.

WS.C1.01.02 Phenotypic, molecular and functional characterisation of pro-inflammatory IL-17+ CD8\(^+\) T (Tc17) cells in psoriatic arthritis

K. J. Steff, U. Sretnathan, L. E. Durham, S. Wu, M. L. Ryder, E. Char, B. W. Kirkham, L. S. Taoms\footnote{1}

\textit{1}Centre for Immunology, Cancer and Inflammation (CICICI), Dept Immunology, School of Immunology & Microbial Sciences, London, United Kingdom, \textit{2}Department of Rheumatology, Guy’s & St Thomas’ Hospital, London, United Kingdom.

Introduction: Psoriatic arthritis (PsA) is an inflammatory joint/disease. Genetic associations implying a role for CD8\(^+\) T cells (HLA-B, RNUX3) and the IL-23/17 axis (IL12B, IL23, TRAF6,3PI2) together with the clinical efficacy of IL-17A blockade provide a strong rationale to investigate IL-17A+CD8\(^+\) Tc17 cells in patients with PsA.

Methods: Mononuclear cells were isolated from peripheral blood (PB) and synovial fluid (SF) from patients with PsA. Cells were stimulated ex vivo before phenotypic, transcriptional and functional analysis.

Results: Tc17 frequencies were increased in the SF vs. paired PB. Phenotypically, SF Tc17 cells (predominantly TcRA) expressed skin and gut tissue-homing (CLA/CD49a/β7 integrin) and Type-17 cell associated markers (CCR6/CD161). TCR-sequencing of SF Tc17 cells suggested a polyclonal TCR repertoire, whilst RNA-sequencing revealed a distinct synovial Tc17 transscriptomic signature compared to PB Tc17, SF Th17 or Tc1 cells. Interestingly, Tc17 cells expressed hallmarks of tissue-resident memory T-cells (CD45RA-CR7-CD103+) whilst sorted CD8+CD69+CD103+ Tc1 cells were enriched for IL-17A. Functionally, SF Tc17 cells co-expressed cytolytic molecules granzyme A and B, pro-inflammatory cytokines IFN-γ, GM-CSF, TNF-α, some IL-21 and IL-22, but little anti-inflammatory IL-10.

Conclusion: We describe a novel phenotypic and molecular signature for PsA synovial Tc17 cells. We also demonstrate, to our best knowledge for the first time, the presence of IL-17A-producing Tc17 cells in the PsA joint. Functionally, Tc17 cells exhibit cytolytic potential and express pro-inflammatory cytokines, suggesting these cells are important contributors to the pathogenesis of PsA.

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WS.C1.01.03 Lymphoid neogenesis in kidneys during lups: involvement of CCKR3-expressing T cells

R. Vebaz, C. Le Coz, S. Leomet, F. Monneaux, K. A. Fenton, H. Dumortier\footnote{1}

\textit{1}Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France, \textit{2}Institute of Medical Biology, Tromso, Norway.

Introduction: Tertiary Lymphoid Organs (TLOs) can be observed in pathological situations such as cancer, infections, graft rejection or autoimmune diseases but the mechanisms leading to their formation remain poorly understood. Our laboratory works on lupus, a chronic and systemic autoimmune disease leading to multiple organ failures among which severe kidney injuries. We have evidenced the presence of functional TLOs in the kidneys of the NZB/W spontaneous lupus mouse model (unpublished). The aim of the present study was to explore the early molecular and cellular mechanisms underlying renal TLO development in lupus.

Materials and methods: Young to old diseased NZB/W and age-matched healthy control BALB/c mice were compared and the leukocyte infiltrates present in their kidneys were characterized by flow cytometry, confocal microscopy and qPCR analyses. Results: We demonstrate that TLO development takes place very early during the disease. Small kidney leukocyte infiltrates can be visualized in the absence of glomerular deposits and before the appearance of detectable circulating autoantibody levels. Among the first infiltrating cells, we found a majority of activated-memory T cells expressing the inflammatory chemokine receptor CCKR3. These T cells are likely attracted by the three CCKR3 ligands as we describe that CXCL9, 10 and 11 are produced in the glomeruli of very young NZB/W.

Conclusions: Altogether, our data suggest that TLO neogenesis takes place at a very early stage of disease development and that blocking T cell infiltration in the kidneys could impair TLO development and help preventing kidney dysfunction in lupus.

WS.C1.01.04 Intracapillary immune complexes recruit and activate slan-expressing CD16\(^+\) monocytes in human lupus nephritis

F. Olaru\footnote{1}, T. Döbel, A. Lonsdorf, A. Ek’s, H. Gröne, K. A. Fenton, H. Dumortier\footnote{1}

\textit{1}Department of Dermatology, Heidelberg, Germany, \textit{2}DKFZ, Heidelberg, Germany.

Lupus nephritis is a major cause of morbidity in patients with systemic lupus erythematosus. Among the different types of lupus nephritis intracapillary immune complex (IC) deposition and accumulation of monocytes are hallmarks of lupus nephritis class III and IV. The relevance of intracapillary ICs in terms of monocyte recruitment and activation, as well as the nature and function of these basal monocytes remains largely unknown. We have recently developed a novel model for the in vitro induction of intracapillary IC formation in human tissue specimens (ICi). In this model, normal peripheral blood were taken at 5 time points and incubated with classical CD14\(^+\)CD16\(^-\), intermediate CD14\(^+\)/CD16\(^+\) and classical CD14\(^-\)/CD16\(^-\) monocytes. Within the population of CD16\(^-\) UN-ILA-DR+ leukocytes (non-classical CD14\(^-\)CD16\(^-\) monocytes) our group defined the population of 6-sulfo LacNAc (slanMo) expressing cells. For the early focal form of lupus nephritis (class III) we demonstrated a selective accumulation of the proinflammatory population of 6-sulfo LacNAc (slan) monocytes (slanMo), which locally expressed TNF-α. In flow chamber experiments, as well as in in vivo model of IC-induced glomerulonephritis immobilized ICs induced a direct recruitment of slanMo from the microcirculation via interaction with Fc-gamma receptor IIa (CD16). Interestingly, intravenous immunoglobulins blocked CD16 and prevented cell recruitment. Engagement of immobilized IC by slanMo induced the production of neutrophil attracting chemokine CXC12 as well as TNF-α which, in a forward feedback loop stimulated endothelial cells to produce the slanMo recruiting chemokine CXC11 (fractalkine). In conclusion, we observed that expression of CD16-classes slanMo with a unique capacity to orchestrate early IC-induced inflammatory responses in glomeruli and identified slanMo as a pathogenic proinflammatory cell type in lupus nephritis.
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WORKSHOPS

WS.C1.01.05

Tbet provides advantage to Tregs for homing into type 1 inflammation sites in vivo


T regulatory cells [Tregs] comprise subgroups that respond to microenvironmental cues. One of such is the Tbet- Tregs, which have adapted the master transcriptional factor of T helper 1 (Th1) cells. Although Tbet is considered as a prerequisite for restraining Th1 responses, there are still gaps in our understanding for how. To address this, we imaged Tbet- Tregs across the course of a Th1 response in live lymph node sections of a Tbet-ZsGreen-Foxp3.2RFP reporter mouse during Type 1 inflammation. We showed that unlike Tbet- Tregs, majority of which resided within the T cell zone, Tbet- Tregs localized specifically to the interfollicular zone. We then generated Tbet- Tregs from naive OTI+ Tbet-ZsGreen-Foxp3.2RFP mice, adoptively transferred into C57BL/6 recipients that had been immunized in the footpad with OVA-CFA. We found higher number of OTI+ ZsGreen-2RFP Tregs than neutral OTII Tregs at popliteal lymph node following OVA-CFA injection, suggesting a homing advantage provided by Tbet. Furthermore, we generated bone marrow chimeras of WT-Tbet- mice and found that compartment lymphoid reconstitution was equally. However, we detected fewer Tbet- Tregs in the spleens of P. Chabaudi infected chimeric mice without any defect in CD44 expression and proliferation level indicating that the defect is not due to activation status but homing. Additionally, Tbet+, Tbet- and Tbet- Tregs suppressed ex vivo differenced Th1 proliferation equally, indicating that Tbet is not required for active suppression. Taken together, we propose that Tbet provides homing advantage to Tregs in vivo without potentiating their suppressive ability once the inflammation site is reached.

WS.C1.01.06

Nox2 deficiency in CD4+CD25+Foxp3+ T cells limits angiotensin II-induced hypertension and cardiovascular remodelling


Introduction: Nox2 is the catalytic subunit of a multi-protein complex that generates superoxide and is known to contribute to hypertension and cardiovascular remodelling induced by angiotension II (ANGII). Recent studies showed that Nox2 has cell-specific roles in cardiomyocytes and endothelial cells but its role in T cell subsets is poorly understood.

Methods: We generated a novel mouse line with CD4-targeted Nox2 deficiency (Nox2-CD4Cre) and studied the response to infusion of ANGII (1.1mg/kg/day, 14 days).

Results: As compared to littermate controls (Nox2+/m), Nox2-CD4Cre+ mice showed an increased proportion of CD4+CD25+Foxp3+ Tregs in the heart and aorta at baseline and after ANGII infusion (multiflow cytometry). This was accompanied by a reduction in infiltrating CD4+RORγ+ and CD8+Foxp3+ T, and inhibition of AngII-induced hypertension, heart fibrosis and cardiomyocyte hypertrophy. The protection in Nox2-CD4Cre+ mice was reversed by depleting Tregs with an anti-CD25/PC61 antibody. In vitro studies revealed that Nox2-deficient CD4+CD25+Foxp3- Tregs suppress proliferation of CD4+CD25+Tfeffs more than WT-Tregs, and inhibits the IL-17 production stimulated by co-culture of Tfeffs with antigen presenting cells in the presence of anti-CD3. Nox2-deficient Tregs also had higher levels of Foxp3 and p65/NF-kB, and increased mRNA levels of CTLA-4, CD39, CD73, GITR and CD253, than WT-Tregs. In adoptive transfer experiments, Nox2-deficient Tregs were more potent at inhibiting ANGII-induced hypertension and heart fibrosis than WT-Tregs.

Conclusion: Nox2 deficiency in Tregs limits cardiovascular remodelling induced by ANGII by suppressing infiltration of Tfeffs. These results suggest that targeting Nox2 in Tregs might be a useful approach in cardiovascular disorders.

WS.C1.02.01

Intestinal Secretory Leucocyte Protease Inhibitor expression is increased in pediatric inflammatory bowel disease patients and is unfavorable during murine colitis


Secretory Leucocyte Protease Inhibitor (SLPI) is an NF-κB inhibitor produced by epithelial cells in response to microbial signals. Previously, we have shown that knockdown of SLPI in intestinal epithelial cells elicits NF-kB activation and subsequent chemokine production, indicating that SLPI inhibits epithelial activation. Crucially, upon increased bacterial pressure at the epithelial border, as seen in mice deficient for one of the major colonic mucus, we detected increased intestinal SLPI expression. As inflammatory bowel disease (IBD) is driven by aberrant host microbial interactions, we hypothesized that intestinal SLPI is beneficial or deleterious during intestinal inflammation.

Results: In therapy-naïve IBD patients, we observed increased SLPI mRNA and protein expression in macroscopically inflamed intestinal tissue, compared to macroscopically non-inflamed tissue, but the expression was not associated with clinical disease activity.

Conclusion: SLPI expression is increased in pediatric IBD patients and is unfavorable during murine colitis.

WS.C1.02.02

ATF3 is crucial for intestinal mucosal immunity during homeostasis and stress

D. Glad; Institute of Biomedical sciences, Taipei, Taiwan.

Activating Transcription Factor 3 (ATF3) is induced by a wide-range of cellular stresses, and found to be involved in many critical human diseases including cancer, atherosclerosis, infections, cardiac hypertrophy, and hypoxias. Interestingly, a recent mouse study has also identified ATF3 up regulation in patients with active inflammatory bowel disease (IBD). Using mouse model and cell system, we have found that ATF3 is crucial to intestinal homeostasis at steady state and protection during inflammation. This was shown, in naive ATF3-deficient mice, by decreased crypts numbers and colon-length indicating defective cellular stemness and regeneration to maintain a healthy tissue mass. Using DSS-induced colitis, ATF3-deficient mice showed lethargy disease activity characterized by a dramatic loss of epithelial architecture and crypt structure, poorness of proliferation/repair machine, and stenuous apoptosis, which has been effectively ameliorated by rectal transplantation of wild-type organoids indicating that the intestinal protective role of ATF3 is mainly occur through maintaining epithelial cell integrity. Paneth cells and stem cells form a niche to orchestrate the differentiation, proliferation, and repair in the intestine. Using electron microscopy, we have identified loss/degeneration of Paneth cell granules, which are the main source of antimicrobial peptides (AMPs), indicating malfunctioning of intestinal Paneth/Stein cell network. Impaired cell proliferation rate and wound healing, and loss of AMPs production have been found also be consistent in ATF3-/- and protective cell line. Collectively, our findings suggest a new insight for ATF3 in terms of intestinal mucosal immunity; a favorable role that brings ATF3 to be a potential competent for further IBD-related researches.

WS.C1.02.03

The transcription factor MAF regulates homeostasis in colonic T cells


1University of Lausanne, Epalinges, Switzerland; 2Lausanne University Hospital, Lausanne, Switzerland.

MAF encodes for a transcription factor belonging to the AP-1 family. In CD4 T helper cells (Th1), it has a role in i-F4 transcriptional regulation in Th2, in Th17 cells through the regulation of Il-10 and Il-23 expression and in Th cells together with the transcription factor bcl6. We recently demonstrated that maf is induced in CD4 and CD8 T cells in melanoma, leading to a “dysfunctional” state of the cells. The role of maf in regulatory T cells (Treg) is less clear. A subset of Treg cells expressing both Foxp3 and RORyt has been described. Present in the gut, RORyt+ Treg cells have an enhanced suppressive activity compared to RORyt- Treg cells, especially in an in vivo context of gut inflammation. Transcriptomic analysis of this population showed an enriched expression of maf in this specific Treg subset. To precise the function of maf in T cells in vivo, we studied a T-cell specific K0 of maf. These mice developed late onset colitis correlating with the loss of RORyt+ Treg cells. While Il-10 expression was reduced, Tnf-α and Il-17A production was increased in the colon. We found both in vitro and in vivo that Maf KO Treg cells produced less Il-10 and had impaired suppressive capacity compared to those derived from WT animals. Our data shows that Maf expression in T cells, especially RORyt+ Treg cells, is essential for their differentiation and to maintain their suppressive activity and thereby prevent inflammatory bowel disease by inhibiting Th1 and Th17 polarization.
The human fetus is thought to be protected from contact with environmental antigens prior to birth, yet evidence has emerged that CD45RO+ T cells are present in the human fetal intestine. We applied a combination of mass cytometric analysis, single-cell sequencing, and functional studies to gain comprehensive insight into the heterogeneity and functionality of the CD4 T cell compartment in the human fetal intestine. Using mass cytometry, we distinguished 22 distinct CD4 T cell clusters, including naïve-like, regulatory, memory-like and CD45RO+CD127+ subsets. Single-cell RNA sequencing confirmed the presence of these distinct CD4 T cell subsets and further characterized their expression profiles. Strikingly, the majority of CD4 T cells were CD45RO+ memory-like cells, expressing high levels of CD69, CD226, CXCR3,CCR4, Ki67 and CD62L. The latter two markers are indicative of cell proliferation and high T cell receptor avidity, respectively. Pathway analysis revealed a strong enrichment for transcripts associated with T cell activation and regulation of the inflammatory response to antigens. In addition, these memory-like T cells showed signs of clonal expansion and were readily stimulated to produce high levels of IFN-γ and some IL-17. Our data therefore provide evidence for the existence of a large pool of memory-like, high avidity CD4 T cells with a proinflammatory phenotype in the human fetal intestine, indicative of antigenic stimulation in utero.

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1

**WORKSHOPS**

**WS.C1.02.04**

Proinflammatory, high avidity CD4 T cells with a memory-like phenotype in the human fetal intestine


1Leiden University Medical Center, Leiden, Netherlands, 2Delft University of Technology, Delft, Netherlands, 3Cardiff University School of Medicine, Cardiff. United Kingdom.

2

**WS.C1.02.05**

The transcription factor NFATc3 promotes intestinal inflammation by suppression of regulatory T cells

K. Gerlach, V. Papp, M. Neurath, B. Weigmann

Medizinische Klinik I, Erlangen, Germany.

The transcription factor NFATc3 (nuclear factor of activated T cells) belongs to a transcription factor family of five members. NFATc3 plays an important role in the activation and function of T cells regulating cytokine production and cell proliferation. High numbers of NFATc3+ cells in the lamina propria of patients suffering from inflammatory bowel disease (IBD) point out the regulatory role of this transcription factor in mucosal inflammation and led us to investigate its function in colitis. Deficiency of NFATc3 in the oxazolone-induced colitis model suppresses induced infection of intestinal inflammation. Staining for Caspase-3 showed less proapoptotic cells in the colon of NFATc3 KO mice whereas apoptotic cells were significantly increased. Additionally, we found a higher number of FoxP3+ T cells in the colon of NFATc3 KO mice suggesting that NFATc3 controls regulatory T cells. As Tregs have been shown to prevent and cure intestinal inflammation caused by the adoptive transfer of naïve T cells in immunodeficient Rag1 knockout mice, we next analysed the relationship between Tregs and NFATc3 in this colitis model. Mice receiving NFATc3-deficient naïve T cells had a later onset of inflammation than mice reconstituted with wildtype T cells. Moreover, the adoptive transfer of NFATc3-deficient T cells was accompanied by an increased number of CD3 FoxP3+ expressing Tregs. In summary, the transcription factor NFATc3 crucially promotes intestinal inflammation by affecting FoxP3 expression and therefore serves as a potential target for therapy in IBD.

**WS.C1.02.06**

The molecular composition of IgA anti-citrullinated protein antibodies in Rheumatoid Arthritis, point to a mucosal origin

M. A. M. van DeRhee*, M. K. Verheul†, N. Levart†, L. Hafkenscheid†, T. Kissel†, A. Bond†, T. W. Huizingo†, K. E. Taes†, L. A. Trouw†

1Miss, Leiden, Netherlands, 2Mr, Leiden, Netherlands.

The presence of autoantibodies targeting post-translationally modified proteins, such as citrullinated (ACP) and carbamylated (anti-CarbP) antibodies, are a hallmark of RA. As these autoantibody responses target homologous structures (citrulline and homo-citrulline) that differ only in one CH group, the IgG ACPA- and anti-CarbP- responses are partially cross-reactive in some, but not in other patients. Both responses use a broad spectrum of isotypes, including IgM, IgG and IgA. To better understand the nature of these prominent autoantibodies, we investigated the molecular composition of both ACPA and anti-CarbP antibodies. Moreover, the degree of cross-reactivity of these autoantibody responses was investigated. Sera of anti-CarbP and/or ACPA positive RA-patients were fractionated using size exclusion chromatography and tested by ELISA for anti-CarbP, ACPA and total IgA and IgG.

Inhibition studies were performed to investigate the relationship between anti-CarbP and ACPA. Our results show that anti-CarbP antibody, rheumatoid factor and anti-E.Coli IgA are predominantly as polymeric IgA, whereas ACPA and anti-Tetanus Toxoid IgA are mostly present as monomeric-IgA. These data are intriguingly as they indicate that the anti-CarbP and ACPA IgA responses are differentially regulated and potentially of different origin. About 75% of the anti-CarbP IgA response cannot be inhibited by citrullinated antigens and vice versa for ACPA IgA by carbamylated antigens, indicating that these autoantibodies are directed against different antigens. To conclude, our data indicate that the anti-CarbP- and ACPA-autobodies responses are differently regulated and may have a different origin, possibly a mucosal associated origin for anti-CarbP antibodies.

**WS.C1.03.01**

Cytokine and transcription factor mediated immune regulation

**WS.C1.03.02**

Anti-TNF treatment leads to delayed activation, maturation and proliferation of CD4+ T cells but does not confer a global suppressive phenotype

G. A. M. Pavlović†, S. Laluninhili, K. Steel, S. Agrawal†, M. Ridley†, S. Kordasti, C. Robert†, L. S. Taams†

†Centre for Inflammation Biology and Cancer Immunology (CIBCI), Dept of Inflammation Biology, School of Immunology & Microbial Sciences, King’s College London, London, United Kingdom.

We previously demonstrated that in vitro treatment of human fetal T cells with the TNF-α-blocking drug adalimumab, promotes anti-inflammatory IL-10 expression. We investigated whether this effect is accompanied by changes in cellular activation, maturation, proliferation and suppressive function. CD4+ T cells from healthy volunteers were cultured for up to 7 days with anti-CD3/CD28 mAb stimulation, in the absence or presence of anti-TNF. Phenotypic changes were evaluated by flow cytometry and CyTOF. Gene expression changes were evaluated using existing datasets (GSE51540). For suppression assays, cells were re-isolated and added to responder T cells, monocytes or fibroblasts. Culturing CD4+ T cells with anti-TNF led to decreased activation as shown by reduced frequencies of CD25+, CD69+ and HLA-DR+ cells. CD4+ T cells also contained significantly higher CD45RA+ and lower CD45RO+ frequencies in the presence of anti-TNF. Proliferation was reduced as indicated by lower percentage of Ki67+ cells and proliferation assays.

Additionally, gene expression analysis of anti-TNF-treated IL-17 or IFNγ-producing CD4+ T-cells revealed multiple pathways associated with cell proliferation and cell cycle. Kinetics experiments suggested that anti-TNF treatment leads to delayed, rather than impaired T cell activation. Furthermore, while anti-TNF treated CD4+ T-cells did not exhibit a significant difference in suppression of T cell proliferation and monocyte production, compared to untreated cells, they displayed hypersuppressiveness, induced the IL-10 regulated molecule CD163 on monocytes and downregulated IL-8 production by synovial fibroblasts. We demonstrate that anti-TNF treatment resulted in delayed activation, maturation and proliferation of CD4+ T cells, but not in acquisition of a global suppressive phenotype. Funded by Arthritis Research UK (RT2119).

**WS.C1.03.04**

Vγ6+ Vδ1 T cells home to the male reproductive tract and expand to populate byways to keep pathways open

H. Briggs*, A. Willham†, S. Sandrock†, T. Amador†, T. Carvalho†, A. Reinhardt†, B. Silva-Santos*, I. Pinz‡**, I. Ribó**

1Instituto de Medicina Molecular Joao Labo Antunes, Lisbon, Portugal, 2Institute of Immunology, Hannover, Germany.

Vγ6+ Vδ1 T cells populate multiple tissues where they have also contributed to the male, but not in the male, reproductive tract. Here, we found that Vδ6 T cells infiltrate the stromal tissue of the testis of naïve C57BL6 mice, expand at puberty and persist throughout life. Strikingly, this population of testicular Vδ6 T cells selectively displayed a Vδ6+ repertoire and was highly enriched in IL-17 producers (Vδ17). In fact, Vδ6 T cells were the major source of IL-17, whereas αβ T cells mostly provided IFNγ in situ. Vδ17 T cell homeostasis in the testsis seemingly depended on IL-1a/IL-23 signals downstream of TLR4 expressed by resident myeloid populations. Interestingly, recent studies have shown that androgens shape the gut microbiome at puberty. Our data suggest that cues from the microbiota may drive the expansion of Vδ17 T cells in the testis, as Germ-Free mice display a significant reduction in this population. Furthermore, we could induce an early Vδ17 T cell expansion in the testsis, before puberty, through adult male fecal transfer. We further hypothesized that testicular Vδ17 T cells might contribute to tissue surveillance. We performed testicular inoculation of Listeria monocytogenes, a commonly used model of orchitis. Our data indicate that infected TCRVδ1-/- and IL-17-/- mice display higher bacterial load and die within 4 days after infection, whereas WT controls survive. Altogether, our results identify a previously unappreciated resident testicular Vδ17 T cell subset that plays a crucial role against local bacterial infection.
WS.C1.03.03
IL-27 increases the plasticity of differentiated Th17 cells by inducing IL-12R beta2 expression

A. Awasthi1, V. E. Kuchroo2
1Translational Health Science & Technology Institute, Faridabad, India, 2B Brigham and Womens Hospital, Harvard Medical School, Boston, United States.

The role of Th17 cells in inducing tissue inflammation in autoimmune diseases is well established. IL-23-IL-23R-induced pathogenic Th17 cells are critical in inducing tissue inflammation in experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis. Interleukin (IL)-27, an IL-12 family cytokine, has been shown to suppress tissue inflammation in EAE partly by inhibiting differentiation of Th17 cells. In addition to inhibition the differentiation and functions of Th17 cells, IL-27 also found to induce the differentiation of IL-10 producing regulatory T cells (Th11), which are found to play an essential role in inhibiting effector T cell functions in EAE. Although the anti-inflammatory role of IL-27 was established, the precise mechanism by which IL-27 suppresses the effector functions of differentiated Th17 cells is not well understood. Using the global gene profile data, we now show that IL-27-induced expression of IL-12Rβ2 plays a critical role in regulating the generation and functions of Th17 cells in EAE. Moreover, IL-27-induced IL-12Rβ2 make differentiated Th17 cells responsive IL-12-mediated conversion into IFN-γ producing T cells. IL-27 receptor deficiency leads to the inhibition of IL-12Rβ2 while enhancing the expression of IL-23R on Th17 cells, and therefore increases the IL-23 responsiveness of Th17 cells. Moreover, IL-27 is unable to inhibit Th17 cell development of experimental autoimmune encephalomyelitis (EAE) in IL23R−/− mice. Indeed, IL-27-induced expression of IL-12Rβ2 increased responsiveness of Th17 cells to IL-12. This provides a novel mechanism by which IL-27 inhibits Th17 cells by increasing their plasticity and responsiveness to immunosuppression.

WS.C1.03.04
TGF-β bioactivity in Trichuris muris homogenate induces FOXP3+ T regulatory cells and inhibits Th1 and Th2 polarization when activated

A. E. Ogunkanbi1, B. Eldahakhy2, J. L. Pennock1
1Institute of Infection, Immunity and Respiratory Medicine, Manchester, United Kingdom, 2Faculty of Medicine, Department of Clinical Biochemistry, King Abdulaziz University, Saudi Arabia, Saudi Arabia.

The establishment of a long lasting chronic infection with helminths relies on their ability to modulate host protective immune responses by direct induction of host immunomodulatory molecules such as IL-10, Tregs cells and TGF-β. Immune modulation by helminths has been centred on induction of host regulatory responses. However, research evidence has shown that helminths themselves can encode TGF-β receptor ligands to modulate the immune response and enhance their survival. We addressed whether T. muris can encode TGF-β like ligands to enhance their survival in the host using bioinformatics, in vitro and in vivo approaches. In vitro, acid treated worm homogenate activates Th1 and Th2 specific cytokine lines. Acid activated worm homogenate also induces foxp3 expression in mouse T cells in a dose-dependent manner and reduces IL-13 and IFN-γ production during T cell polarisation. The induction of foxp3 was abolished by anti TGF-β antibody (1D11) and both the production of IL-13 and IFN-γ was restored upon addition of 1D11. Finally, T. muris homogenate can induce eosinophilic foci of TGF-β both in vivo and in vitro and this associated with the reduction in IFN-γ production. These data have shown that unique TGF-β activity is present in T. muris and supports the current paradigm that worms have evolved mechanisms to potentiate their survival. These data also complement and extend our current understanding of helminth immunoregulation and broaden the scope for potential therapeutics for regulation of intestinal inflammation.

WS.C1.03.05
Hobit identifies precursors of resident memory T cells within the peripheral tissues

L. Parga Vidic, F. Behr1, N. Kragneti, T. Wesselin, R. Stark2, K. van Gisbergen2
1Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, AMC, University of Amsterdam, Amsterdam, Netherlands, 2Department of Experimental Immunology, AMC, Amsterdam, Netherlands.

Tissue-resident memory T (Trm) cells constitute a non-circulating memory subset that provides early protection against re-infection. It is unclear when, where and how these Trm develop from effector CD8 T cells. We have previously described that Hobit represents a Trm-specific transcription factor that is essential for their formation. To employ Hobit for the study of Trm differentiation, we generated a Hobit reporter mouse, which contains a “knock-in” of the fluorescent protein tdTomato and the diphtheria toxin (DT) receptor within the Hobit locus. We infected Hobit reporter mice with LCMV and analyzed virus-specific CDB T cells for tdTomato expression by flow cytometry. Trm present in gut, kidney, liver and salivary glands nearly uniformly expressed tdTomato in contrast to circulating memory. Interestingly, tdTomato was already upregulated in a subset of effector cells located within these peripheral tissues, but not in lymph nodes, blood or spleen. To examine the potential of the tdTomato+ effector cells to establish memory populations, we depleted them using DT injections at different time-points after infection. We observed a substantial and specific decrease in Trm after depletion of tdTomato+ effector cells between day 7 and 10 after infection. Depletion at earlier time points did not have an impact on Trm formation. These findings show that Hobit+ effector CD8 T cells are Trm precursors. Furthermore, we conclude that commitment of effector T cells to the Trm lineage occurs in the peripheral tissues at the peak of the effector CD8 T cell response.

WS.C1.03.06
EOMES-positive CD4+ T cells are increased in PTPN22 (1858T) risk allele carriers

K. Chemin1, D. Ramskdil2, L. Diaz-Gallo1, J. Herratti1, M. Oufmany1, K. Tandre1, L. Rönnblom3, V. Malmström4
1Center for Molecular Medicine, Stockholm, Sweden, 2Uppsala University, Uppsala, Sweden.

The presence of the PTPN22 risk allele (1858T) is associated with several autoimmune diseases including rheumatoid arthritis (RA). Despite a number of studies exploring the function of PTPN22 in T cells, the exact impact of the PTPN22 risk allele on T-cell function in humans is still unclear. In this study, using RNA sequencing, we show that, upon TCR-activation, naive human CD4+ T cells homoygous for the PTPN22 risk allele overexpress a set of genes including CCL4 and 4-1BB, which are important for cytotoxic T cell differentiation. Moreover, the protein expression of the C box transcription factor Eomesodermin (EOMES) was increased in T cells from healthy donors homozygous for the PTPN22 risk allele and containing the highest levels of VLA-4, and selectively accumulated in natalizumab-treated MS patients who remained stable over time. EOMES+ CD4+ T cells were more frequent in natalizumab and TNF-α treated patients compared to TNF-α treated patients without natalizumab therapy. These findings provide a novel mechanism by which PTPN22 risk allele influences T cell differentiation and function.

WS.C1.04.04
Regulation in tissue specific autoimmune 2

WS.C1.04.01
Th17.1 cells preferentially recruit to the central nervous system to mediate early disease activity in multiple sclerosis

J. van Langelaar1, R. M. van der Vuurt de Vries2, M. Janssen1, A. F. Wierenga-Wolff2, J. A. Spitteler3, T. A. Siepman4, W. Dankers2, T. A. Siepman4, W. Dankers2, R. O. Hintzen1, M. M. van Luijn1
1Erasmus MC, Rotterdam, Netherlands, 2MS Center ErasMS, Rotterdam, Netherlands, 3University of Veterinary Medicine, Hannover, Germany, 4VU University Medical Center, Amsterdam, Netherlands.

Multiple sclerosis (MS) is mediated by pathogenic CD4+ T cells that infiltrate the CNS to promote local inflammation and demyelination. IL-17-producing CCR6+ Th cells are the main drivers of EAE, the animal model for MS. However, the functional contributions of CCR6+ Th cells are heterogeneous and differ between mice and men. Here, we assessed distinct effector populations of human Th17 cells and how their recruitment to the CNS associates with MS disease onset. Low frequencies of CRC6+CXCR3+ (Th1-like Th17), and not CRC6+CXCR4+ (Th17) effector memory cells in the blood strongly associated with rapid diagnosis of MS. In CSF, Th1-like Th17 cells were abundant and showed increased IFN-γ/GMN-CSF production compared to paired CCR6+ and CRC6- Th17 cells and their blood equivalents after short-term culturing. Their local enrichment was confirmed ex vivo using paired MS CSF and brain single-cell suspensions. Across all pro-inflammatory Th17 populations analyzed in the blood, a IL-17−/IFN-γ−/GM-CSF− subset termed Th17.1 (CCCD5/CXCR3/CXCR4−) expressed the highest levels of IL-6, IL-10, and selectively accumulated in natalizumab treated MS patients who remained afebrile of acute relapses. This was not found for patients who encountered relapses. The pathogenicity of Th17.1 was further supported by their predominance in early MS CSF, enhanced transmigration across human brain endothelial monolayers in vitro, and increased expression of IL-23R, MDR1 and granzyme B. These findings reveal a selective contribution of Th17.1 cells to CNS inflammation and provide a strong rationale for more refined and earlier use of T cell-directed therapy in MS patients.

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**WORKSHOPS**

**WS.C1.04.02**

4-1BB overexpression in basal keratocinocytes induces the disruption of the eye’s immune privilege

E. Gonnelli, M. Kasper, H. Kupsa, D. Bauer, T. A. Luger, A. Heiligenshmugl, K. Lasser;

1Department of Dermatology, Münster, Germany, 2Institute of Experimental Ophthalmology at Franziskus Hospital, Münster, Germany, 3MorphoSys AG, München, Germany.

4-1BB (also called CD137 or TNFRSF5) belongs to the tumor necrosis factor receptor superfamily (TNFRSF), and has a crucial role as a costimulatory molecule in a variety of immune processes. The overall effect of 4-1BB/4-1BB ligand signaling is enhancing inflammatory responses. To investigate the role of 4-1BB in more detail we generated a mouse model with overexpression of 4-1BB under control of the keratin-14 (K-14) promoter. Surprisingly, besides severe pruritus, K14-4-1BB tg mice spontaneously developed uveitis and anterior cataract beginning at the age of 3 weeks, which was associated with the infiltration of immune cells into the eye, finally resulting in blindness. Immunofluorescent staining as well as qPCR and FACS analysis was used to characterize the cell infiltrate, revealing that the infiltrate was mainly consisting of MCHCII+4/BD+ macrophages. Moreover, by performing whole mRNA array analyses we confirmed the downregulation of genes related to tight junction formation, cell-cell interaction and transmigration that might favor the in vivo recruitment of immune cells into the eye. Interestingly, we also observed the presence of markers supporting an epithelial-mesenchymal-transition (EMT) occurring in the epithelial layer of the anterior chamber’s lens capsule from K14-4-1BB tg mice strongly suggesting that the EMT might be the trigger for the infiltration of immune cells through leaky cell junctions. Together, these data indicate that 4-1BB signaling is crucially involved in the development of uveitis and anterior cataract and might play an important role in disrupting the immune privilege of the eye.

**WS.C1.04.03**

Targeting CD146 can downregulate new blood vessel formation caused by VEGF-producing T cells in rat experimental autoimmune uveitis

G. Wildner, M. Diedriechs-Möhning, S. Thuraus;

Section of Immunobiology, Department of Ophthalmology, University Hospital, LMU Munich, Munich, Germany.

Neovascularization in the retina as a consequence of inflammation is a major problem in the eye. We have recently shown that VEGF-producing autoreactive T cells in rat experimental autoimmune uveitis can also induce choriotelial neovascularization (CNV). Here we investigate the expression of CD146, a molecule of endothelial tight junctions and a coreceptor of VEGFR-2, in the eye and the effect of antibody to CD146 on uvea and formation of CNV. To induce monocular choroidal uveitis neovascularization Lewis rats were immunized with S-Ag peptide PDSAg-CA or adoptively transferred with PDSAg-specific T cells. After adoptive transfer anti-CD146 antibody was daily injected s.c. and uveitis intensity monitored clinically and histologically. To investigate the effect on CNV formation, anti-CD146 antibody was injected once into the anterior chamber of rats one day prior to onset of clinical disease and CNV formation determined histologically. Cryosections from rat eyes with uvea were stained with anti-CD31/PECAM and anti-CD146. In eyes with uvea CD146 was expressed in the choroid/choricapillaris and CNV, also on some infiltrating T cells. Preventive systemic or intraocular treatment with anti-CD146 only marginally affected the intensity of intraocular inflammation, while a single intraocular injection of anti-CD146 prior disease onset significantly suppressed CNV formation. Anti-CD146 antibodies can prevent the pathological development of new vessels in the eye after a single intraocular injection in an EAU model, where autoreactive T cells produce VEGF and induce CNV. Targeting this molecule could be a new therapeutic option to prevent the growth of new blood vessels.

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**WS.C1.04.04**

Persistence of dominant TCRα clones in regulatory T cells derived from an autoimmune inflammatory environment

G. Mijnheer, J. Leong, A. Boljesi, E. Spierings, A. Petrelli, S. Vasterl, S. Albani, A. Pandi, F. van Wijk;

1University Medical Center Utrecht, Utrecht, Netherlands, 2SingHealth and Duke-NUS Graduate Medical School, Singapore, Singapore, 3SingHealth and Duke-NUS Graduate Medical School, Singapore, Singapore.

Inflammation is characterized by infiltration of multiple immune cell types and expansions of antigen-specific T cells. In autoimmune diseases, inflammation is often limited to specific target tissues, but within these sites, multiple sites can be affected. An important outstanding question is whether affected sites are infiltrated with the same pathogenic T cell clones and whether these clones persist over time. In Juvenile Idiopathic Arthritis (JIA) it is relatively easy to analyze cells derived from the site of inflammation, i.e. inflamed joints. Here we performed CyTOF and T cell receptor (TCR) sequencing to study immune cell composition and hyperexpansion of inflamed joint derived T cells. The samples were taken from different joints affected at the same time, and joints that were affected multiple times during the relapsing remitting course of the disease. CyTOF analyses revealed that the composition and functional characteristics of the immune infiltrates are strikingly similar between joints within one patients. Furthermore we observed a strong overlap between dominant T cell clones (Teff and even more pronounced for Treg) in inflamed joints affected at the same time, and some of the most dominant clones could also be detected in circulation. Finally, these dominant T cell clones were found to persist over time and to expand during relapses, even after full remission of the disease. These data suggest that in autoimmune disease there is auto-antigen driven expansion of both Teff and Treg clones, that are highly persistent and are re-circulating. Therefore these dominant clones can be interesting therapeutic targets.

**WS.C1.04.05**

Characterization of constitutively DC-deficient mice in autoimmune responses

C. Hilpert, D. Voehringer;

Department of Infectionbiology, Erlangen, Germany.

Introduction: Dendritic cells (DCs) play an important role as antigen-presenting cells for T-cells, but they can also induce T-cell tolerance and protect against autoimmunity. We recently reported the generation of constitutively DC-deficient mice (ΔDC mice) which can be used to address the specific function of DCs in vivo. These mice lack 95% of classical DCs, the majority of plasmacytoid DCs and Langerhans cells. ΔDC mice show impaired negative thymic selection, hyperimmunglobulinemia and autoantibody production. About 40% of these mice develop severe autoimmune pathology starting at 6-8 weeks of age.

Objectives: We are investigating the cellular mechanisms which lead to an adoption and autoimmunity in ΔDC mice.

Methods and results: We observed that mice lacking DCs have reduced numbers of regulatory T cells (Treg) in the gut. Microarray and flowcytometry analysis showed that those Treg show a higher expression level of inhibitory surface receptors. Analysis of suppressive function of Treg from ΔDC mice in vitro assays revealed that Treg from ΔDC mice are less suppressive, but Treg from both strains suppress equally well in an adoptive transfer model of colitis with Rag-ko recipients. Using DC-deficient Rag-ko mice as recipients, we could see that Treg from both strains show impaired function. By creating mixed bone marrow chimeras where DCs lack MHC-II we observed weightloss, intestinal inflammation and an expression pattern of inhibitory molecules on Treg similar to ΔDC mice.

Conclusion: From our studies we can conclude that contact to DCs via MHC-II is required for proper Treg function and prevention of severe autoimmune inflammation.

**WS.C1.04.06**

Engineering antigen-expressing regulatory T cells to modulate adverse immune responses

D. W. Scott, M. Abdelalim, A. Zhang, J. H. Yoon, K. Parvathanneni, L. Kropel, Y. C. Kim, E. Mitre;

Uniformed Services University of the Health Sciences, Bethesda, United States.

Expanded regulatory T cells (Tregs) have been proposed for the treatment of adverse immune responses to biotherapeutics, autoimmunity, transplantation and allergy. To increase efficacy and specificity of Tregs, we previously engineered human and mouse T cells to express chimeric antigen receptors (CARs) by expressing either T-cell receptors (TCR) from human T cell clones (derived from patients) or specific single chain fragments (scFv). All of those engineered specific Treg cells actively suppressed effector antibody responses in vitro and in vivo and modulated EAE. Recently, we expanded this approach to create antigen-specific cytotoxic T cells as well as Tregs expressing antigenic domains (B-cell antibody receptors or BARs). Such BARs are able to kill antigen-specific hybridomas and LPS-activated normal B cells in vitro. In one model, BAR Tregs expressing cloting Factor VIII (FVIII) actively and specifically suppressed an antibody response to FVIII in vivo. BAR Tregs expressing ovalbumin (OVA) modulated the anaphylactic response (as monitored by temperature drops) to OVA. These results provide a novel paradigm to specifically regulate harmful immune responses in patients. (Supported by grants from the NIH)
Induction of gladin-specific immune inhibition following treatment with Tolerogenic Immune Modifying Nanoparticles (TIMP) containing gladin

J. R. Pedraza1, T. L. Freitag2, R. Pearson3, D. Getts4, L. D. Sheil1, S. D. Miller1

1Northwestern University, Chicago, United States; 2COEUR Pharmaceuticals Development Company, Chicago, United States; 3University of Helsinki, Helsinki, Finland; 4University of Michigan, Ann Arbor, United States.

In celiac disease, tolerance to gluten proteins from cereals is lost. Tolerogenic Immune Modifying Nanoparticles (TIMP) are poly(lactide-co-glycolide) that contain autoreactive protein or peptide epitopes. These nanoparticles have been shown to induce immune tolerance in numerous autoimmune conditions. The identification of gladin as the primary epitopes in celiac disease suggest that TIMP containing gladin may serve as a tool to induce tolerance to gladin and potentially cure celiac disease. Here we developed gladin containing TIMP, referred to as TIMP-GLIA, and examined its safety and ability to induce immune tolerance in a delayed type hypersensitivity (DTH) and celiac disease animal model setting. The present data demonstrate the safety and preclinical efficacy of TIMP-GLIA to restore gluten tolerance. In vitro studies demonstrated TIMP-GLIA were compatible with intravenous infusion in humans, induced a tolerogenic phenotype of human dendritic cells, and did not trigger T cell activation when incubated with PBMCs from active CD patients. In three mouse models of celiac disease, 1) a delayed-type hypersensitivity, 2) a HLA-DQB transgenic, and 3) an adoptive gladin memory T cell transfer model, treatment with TIMP-GLIA resulted in antigen-specific reductions in T cell proliferation, inflammatory cytokine secretion, circulating gladin-specific IgG/IgG2c, gluten-dependent enteropathy, and body weight loss. The results provide preclinical support for the safety and efficacy of TIMP-GLIA treatment in re-establishing peripheral immune tolerance in CD patients.

Inhibition of arginase-1 expression by the transcription factor Fra-1 in inflammatory macrophages exacerbates rheumatoid arthritis inflammation

N. Hannefmann1, S. Cao2, A. Schneiter3, J. Jordan1, M. Eberhardt4, U. Schliecher5, S. Uebel1, A. Ekiç6, J. Rech7, T. Bäuerle1, C. Bogdan1, J. Vera8, G. Schett8, A. Bazec1

1Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Department of Internal Medicine 3 – Rheumatology and Immunology, Universitätsklinikum Erlangen, Erlangen, Germany; 2Institute of Radiology, Preclinical Imaging Platform Erlangen (PIPE) Universitätsklinikum Erlangen, Erlangen, Germany; 3Department of Dermatology, Laboratory of Systems Tumor Immunology Universitätsklinikum Erlangen, Erlangen, Germany; 4Friedrich-Alexander-University Erlangen-Nuremberg (FAU), Institute of Microbiology – Clinical Microbiology, Immunology and Virology, Universitätsklinikum Erlangen, Erlangen, Germany; 5Institute of Human Genetics Universitätsklinikum Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany.

The activator protein (AP)-1 transcription factor family, especially its subfamily of FOS proteins (cFos, FosB, Fra-1 and Fra-2), are associated to the regulatory network of macrophage responses. Macrophages are central player during rheumatoid arthritis (RA). This study aims to delineate the role of Fra-1 in macrophages during the acute disease and inflammatory phase of RA. Therefore, we applied the serum-induced arthritis (K/BxN) model to Fra-1 deficient mice controlled by the Mxl promoter (Fra-1−/−) or the LysM promoter (Fra-1lox/lox). Fra-1 mutant mice had decreased arthritis severity compared to their littermate wildtype mice. The alleviated arthritis was accompanied to increased arginase-1 (Arg1) expression and activity in the joints, which was increased in Fra-1 mutant mice. Mechanistically, chromatin immunoprecipitation (ChIP) sequencing and conventional ChIP, a novel chromatin immunoprecipitation reporter analysis uncovered, that Fra-1 transcriptionally inhibited Arg1 expression in macrophages. Moreover, inhibition of Arginase in Fra-1 mutant mice restored a full-blown inflammatory RA response and the supplementation of mice with L-arginine, leading to increased arginase activity in the joint, is sufficient to milder arthritis. Synoviom histological sections from RA patients showed a correlation between Arg1, Fra-1 and the DAS28 score, confirming that increased Arg1 activity is of benefit also for human inflammatory joint disease. Our data show for the first time that Fra-1 is a pivot between pro- and anti-inflammatory macrophage. By inhibiting Arg1 activity, Fra-1 exacerbates RA inflammation and joint destruction.

OPAL1, a novel transmembrane adaptor protein regulates CXCR4 signalling

Š. Born1, A. Drobek, J. Králová, M. Fabišík, T. Brdička

1Institute of Molecular Genetics, Prague 4, Czech Republic.

Spatial distribution of immune cells in the body of vertebrates is essential for proper function of the immune system. Destination of each cell is defined by gradient of chemokines, composition of extracellular matrix, and expression pattern of surface receptors, of which chemokine receptors play the most critical role. We have analyzed the function Outcome Precursor of Acute Leukemia 1 (OPAL1) a transmembrane adaptor protein with unknown function that is upregulated in TEL/AML1-positive childhood acute lymphoblastic leukemia and higher expression of which was suggested to be associated with favourable prognosis. Using shRNA-mediated knock down we found that it is a negative regulator of CXCR4 signalling in immortalized murine monocyte progenitors and in human TEL/AML1-positive leukemic B cell line REH. Moreover, OPAL1 knock down in zebrafish changed the spatial organisation of immune cells in the embryo. However, our analysis of OPAL1-deficient mice revealed rather mild phenotype, which is inconsistent with enhanced CXCR4 signalling. Moreover, both splenocytes and bone marrow cells showed normal response to CCL12, a ligand of CXCR4. We speculated that over time these mice can compensate for the loss of OPAL1 function. In accordance with our hypotheses, bone marrow cells from inducible OPAL1 knock out mice have enhanced CXCR4 signalling upon acute OPAL1 deletion. Our studies of the molecular mechanism show that OPAL1 interacts with Nedd4 ligand family members, known regulators of CXCR4 signalling, and suggest that through these interactions OPAL1 modifies CXCR4 signalling.

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Fcγr-TLR cross-talk promotes inflammation by human antigen presenting cells via IRF5-dependent gene transcription and glycolytic reprogramming


1University of Amsterdam, Amsterdam, Netherlands; 2Academic Medical Center, Amsterdam, Netherlands; 3Leiden University Medical Center, Leiden, Netherlands.

Antigen presenting cells (APC) are crucial for initiation of adequate inflammatory responses, which critically depends on the cooperation of different receptors. An important recently identified route of induction of inflammation by APCs involves cross-talk between Toll-like receptors (TLRs), recognizing microbial structures or dead/damaged host cells, and low-affinity Fc gamma receptors (FcγRs), recognizing IgG immune complexes. The physiological function of this FcγR-TLR cross-talk is to provide protective immune responses against invading pathogens. However, undesired activation of Fcγr-TLR cross-talk, e.g. by autoantibodies, also plays a major role in the development of chronic inflammatory disorders such as rheumatoid arthritis (RA). Since interfering with Fcγr-TLR cross-talk may have great therapeutic potential, we set out to identify the responsible molecular mechanisms. Strikingly, we identified that production of pro-inflammatory cytokines by Fcγr-TLR cross-talk critically depends on activation of interferon regulatory factor 5 (IRF5), which amplifies gene transcription and induces metabolic reprogramming. We show that TLRs and Fcγr synergize by inducing two independent signaling pathways that ultimately converge on IRF5 activation. First, TLR stimulation resulted in phosphorylation of TBK1/IKKe, which is required for IRF5 phosphorylation and subsequent activation. Second, we identified that Fcγr stimulation signals via a Syk-dependent pathway to induce IRF5 nuclear translocation for amplification of gene transcription. Additionally, Fcγr stimulation strongly increases the glycolytic rate of APCs, which is also essential for the synergistic induction of inflammation by Fcγr-TLR cross-talk. Taken together, these data provide new potential targets to suppress inflammation in auto-antibody associated diseases such as RA, systemic sclerosis, and systemic lupus erythematosus (SLE).

Transmembrane TNF signaling through TNF-R1 induces SpA-like inflammation, whereas signaling through TNF-R11 is crucial for new bone formation

M. van Tolk1, D. Potz1, J. Blijdorp2, M. Armašek1, G. Kollias1, M. van de Sande2, D. Baeten1, L. van Duivenvoorde3

1Amsterdam Rheumatology and Immunology Center, Amsterdam, Netherlands; 2Biomedical Sciences Research Center “Alexander Fleming”, Vari, Greece; 3Biomedical Sciences Research Center “Alexander Fleming”, Vari, Greece.

Background. TNF can drive distinctly inflammatory pathologies depending on its expression form. We have shown that transmembrane (tm)TNF rather than soluable TNF contributes to pathological features of sporadic arthritis (SpA), including the key hallmark pathological new bone formation. Objective. Delineate the cellular and molecular mechanisms by which selective tmTNF overexpression leads to SpA-like pathology. Methods. tmTNFtg mice (TgA86) were crossed with TNF-R1 or TNF-R11 knockout mice and followed clinically for SpA development. Carvalibro fibroblasts were cultured and differentiated towards osteoblasts. Results. SpA was observed in all tmTNFtg and tmTNFtg×tmTNFtg−/− mice but not in tmTNFtg×tmTNFtg−/− mice and confirmed by histology. Whereas this indicates that TNF-R1 is required for tmTNF-induced inflammation, it was striking that 50% of the tmTNFtg×tmTNFtg−/− mice depicted clear histological signs of endochondral new bone formation. To test whether TNF-R11 is involved in new bone formation, carvalibro fibroblasts from tmTNFtg×tmTNFtg−/−, tmTNFtg×tmTNFtg−/− mice and wild type mice were differentiated with osteogenic medium with or without IL-17A. tmTNF overexpressing fibroblasts enhanced osteogenic differentiation as evidenced by alkaline phosphatase and alizarin red staining and increased mRNA levels of Collagen type I and ALP compared to wild type fibroblasts. This enhancement in osteogenesis was maintained in tmTNFtg×tmTNFtg−/− derived fibroblasts but abolished in tmTNFtg×tmTNFtg−/− derived fibroblasts. Conclusion. The SpA-like phenotype in tmTNFtg mice is crucially dependent on TNF-R1 to drive inflammation, but TNF-R11 signaling is required for new bone formation under inflammatory conditions.
**WORKSHOPS**

**WS.C2.01.06**

Lactate, via SLC5A12, lights up inflammation in CD4+ T cells by inducing a metabolic reprogramming

V. Puccino, M. Certo, D. Cucchi, M. Lewis, K. Goldmann, M. Bombardieri, C. Pitzalis, C. Maurus; William Harvey Research Institute, Queen Mary, University of London, London, United Kingdom.

Introduction: Inflammatory sites are characterised by the accumulation of lactate. CD4+ T-cells sense lactate, via the transporter SLC5A12, which in turn inhibits their motility and promotes their switch to the Th17 subset. We assessed whether the lactate/SLC5A12-induced-metabolic-signalling-pathway is key to the chronic inflammation and autoimmunity. Material and methods: Mononuclear-cells were isolated from tonsils of patients undergoing tonsillectomy from peripheral blood of healthy volunteers and from peripheral blood and synovial fluid of RA-patients. SLC5A12 expression was evaluated by flow-cytometry. RNA-sequencing-analysis was performed for the expression of metabolic genes in synovial tissues of naïve-to-treatments RA-patients. IL17 expression was assessed by RT-PCR and ELISA. Seashore and western-blot analysis were performed for the evaluation of metabolic pathways. IL17-signalling pathway was evaluated by western-blot.

Results: SLC5A12 is up-regulated by CD4+ but not CD8+ T-cells upon triggering of the T-cell receptor (TCR). This expression is higher in CD4+ T-cells isolated from RA synovial fluid, where lactate is more abundant, compared to peripheral RA and healthy donors CD4+ T-cells. Lactate-uptake by CD4+ T-cells through SLC5A12 causes a reprogramming of glycolysis and fatty acids metabolism that tied to migration and cytokine responses via the induction of specific signalling pathways. Antibody-mediated blockade of SLC5A12 limits lactate induced CD4+ T-cells IL-17A and restores lactate-impaired CD4+ T-cell migration by rescuing glycolysis hence leading to beneficial egression of inflammatory CD4+ T-cells from inflamed tissues. In vivo treatment with SLC5A12 blocking antibody ameliorates the clinical course of disease in a mouse model of arthritis.

Conclusions: Targeting lactate/SLC5A12-induced-metabolic-signalling-pathway may provide a novel therapeutic-strategy to reduce inflammation.

**WS.C2.02 Neuroinflammatory disorders**

**WS.C2.02.01**

Efficient suppression of effector T cells isolated from multiple sclerosis patients by autologous, UniCAR-engrafted Tregs

A. Kegleri, S. Koristka, A. Fereczi, E. Gillingham, S. Albert, M. Khameni, H. E. de Vries, C. Serhan; 1Technische Universität Dresden, Tumor Immunology, Dresden, Germany, 2Medical Clinical and Polyclinic I, University Hospital ‘Carl Gustav Carus’, TU Dresden, Dresden, Germany, 3German Cancer Center (DKTK), partner site Dresden, and German Cancer Center (DKFZ), Heidelberg, Germany, 4National Center for Tumor Diseases (NCT), Dresden, Germany, 5TU Dresden, TU Dresden, Dresden, Germany, 6Institute of Immunology ‘Carl Gustav Carus’ and Germany, 3Center of Clinical Neuroscience, Department of Neurology, University Hospital ‘Carl Gustav Carus’, TU Dresden, Dresden, Germany.

Introduction: In multiple sclerosis (MS) patients pathogenic, autoreactive effector T cells (Th17) provoke demyelination and chronic nerve system damage. To impede those harmful immune reactions, the adoptive transfer of regulatory T cells (Tregs) emerged as a promising therapeutic strategy. Several preclinical mouse models confirm an inferior functionality of polyclonal compared to antigen-specific Tregs. However, isolation and expansion of Tregs with a desired antigen-specificity proves to be highly time-consuming and labor-intensive. Methods and Materials: To overcome these hurdles, we armed polyclonal Tregs isolated from MS patients with a universal chimeric antigen receptor (UniCAR) construct. This innovative technology allows a site-specific reprogramming of autoreactive T cells against any desired surface structure, as cross-linkage to target cells is mediated by a separate, antigen-binding targeting module (TM). Results: Highly pure CD4+CD25+CD127lowCD45RA− MS-Tregs could be genetically modified to stably express the UniCAR4-1BB/C-construct. UniCAR-armed Tregs strongly expanded and show phenotypic stability also upon pro-inflammatory challenge. By adding a TM in the presence of target cells, UniCAR-engrafted Tregs are antigen-specifically activated demonstrated by CD69 and LAG upregulation. Most importantly, upon TM-stimulation UniCAR-armed Tregs efficiently suppress pre-activated, patient-derived Th17. Conclusions: Taken together, the UniCAR system holds an enormous therapeutic potential for MS, as it not only allows a site-specific and precisely regulated Treg activation but also confers strong suppressive capacity to Tregs from MS patients. Thereby, this innovative technology might broaden current treatment strategies to overcome impaired functionality of Tregs as well as resistance of pathogenic Th17 to Treg suppression reported for MS patients.

**WS.C2.02.02**

Elucidation of pro-resolving lipid mediators in the cerebrospinal fluid: implications for multiple sclerosis pathogenesis

G. Koellü, V. Chiurchiù, G. Ermann, P. Norris, M. Khameni, B. Engelhardt, H. E. de Vries, C. Serhan; 1VUmc MS Center Amsterdam, Amsterdam Neuroscience, Amsterdam, Netherlands, 2Harvard Medical School, Boston, United States, 3European Center for Brain Research, Rome, Italy, 4Theodor Kocher Institute, Bern, Switzerland, 5Karolinska Institutet, Stockholm, Sweden.

Background and objective: The acute inflammatory response is host protective and efficient resolution of inflammation is required to prevent excessive inflammation and restore tissue homeostasis. This protective process is orchestrated by specialized pro-resolving lipid mediators (SPMs) that are biosynthesized from omega-3 fatty acids. In the chronic neuroinflammatory disease multiple sclerosis (MS), the abundant presence of pro-inflammatory cells and wide-spread microglial activation within the central nervous system (CNS) suggests that this resolution process is impaired. Consequently, the uncontrolled inflammatory response will acquire a chronic nature, leading to severe tissue damage (MS pathology) and disease progression. To date, fundamental insights into the regulation of CNS resolution processes and whether impairments in this system correlate with MS progression remain elusive.

Methods: We used liquid chromatography-tandem mass spectrometry (LC-MS-MS)-based metabololipidomics to reveal lipid mediator signatures in the cerebrospinal fluid (CSF) of RRMS (either in relapse or remission), SPMS and PPMS patients as well as age/sex matched controls. Results: We report the first evidence for resolution of inflammation defects in MS as reflected by a complete absence of brain specific SPMs in the CSF in all MS cases. We also defined the source of these SPMs, their target cells in the CNS during neuro-inflammation and determined their in vivo efficacy in an MS mouse model.

Conclusion: By using metabololipidomic profiling of human CSF, we here provide first evidence that impaired resolution pathways exists in MS and thereby highlight the potential to use SPMs as novel therapeutics as well as biomarkers for diagnosis.

**WS.C2.02.03**

Characterizing human CD20+ T cells and their role in multiple sclerosis

M. von Essen, C. Ammitzbøll, R. Hansen, O. McWilliam, E. Petersen, H. Marquart, F. Sellebjerg; 1Danish Multiple Sclerosis Center, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, 2Department for Clinical Immunology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.

Introduction: Recently we found that T cells expressing CD20 showed high reactivity to central nervous system (CNS) antigens. In addition, the frequency of CD20+ T-cells was increased in blood of patients with multiple sclerosis (MS), suggesting a role in the pathogenesis. In this study, we further characterized the phenotype of CD20+ T-cells and their implication in MS.

Results: Analysing the expression of chemokine receptors and adhesion molecules, assumed to recruit blood T cells to the CNS, showed that the frequency of blood CD20+ T-cells from patients with relapsing-remitting MS (RRMS) expressing CCR2,CCR5,CCR6,CXCR3 and a high level of CD49D was significantly increased compared to CD20- T-cells. This increased CNS migration potential was substantiated by the observation that CD20+ T-cells were enriched in the cerebrospinal fluid (CSF) from patients with RRMS. A role in MS pathogenesis was further supported by a positive correlation between CD20+ T-cells in the CSF and demyelination measured as free myelin basic protein in the CSF as well as a positive correlation with disease severity (EDSS). Characterizing CD20+ T-cells revealed a pro-inflammatory T cell producing high levels of IFN-γ, TNF-α and GM-CSF as well as granulocyte A and K compared to CD20- T-cells; both in the CD4 and the CD8 compartment.

Conclusions: Our data indicate that CD20+ T-cells have a Th1/Th17 phenotype and possibly cytokinolytic activity and that they may play a central role in the pathogenesis of MS.
Dimethyl fumurate limits Tc17 cell fate in autoimmune via ROS accumulation

B. Broux 1, S. Ghorbani, F. Talebi, F. Noorbakhsh 2, I. Jongerius 1, B. M. Luken 2, G. van Mierlo 2, S. Zeerleder 1,2, A. Prat 2, S. Zandee 1,2, M. Lécuyer 2, A. Bouthillier 2, R. Mouamdjia 4, P. Duquette 1, N. Aubari 1, A. Pratt 1
1Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium, 2CRCUM, Montreal, Canada, 3University Medical Centre, Goettingen, Germany, 4University of Pennsylvania, Philadelphia, United States, 5CHUM, Montreal, Canada

In multiple sclerosis (MS), demethylated lesions are caused by an inflammatory response in the central nervous system (CNS). Pathogenic T helper T (Th) 17 CD4+ T lymphocytes are involved in the development of these lesions. Two cytokines expressed by these cells, namely IL17 and IL21, have been shown to disrupt blood brain barrier (BBB) integrity, an important early event in MS lesion formation. Here, we report that IL26 expression is induced in human CD4+ T lymphocytes by T17-inducing cytokines and is significantly upregulated in the blood and cerebrospinal fluid of MS patients. In addition, CD4+IL26+ T lymphocytes are found in perivascular immune infiltrates in MS brain lesions and the two receptor chains for IL26, IL10R2 and IL20R1 are detected on BBB endothelial cells (ECs) in vitro and in situ. Unexpectedly, we found that IL26 promotes tightness and reduces permeability of BBB in vitro and in vivo. Finally, transplanted mice with experimental autoimmune encephalomyelitis (EAE) engrafted with IL26+ T cells showed decreased disease severity and pro-inflammatory cytokine infiltration into the CNS. Our study demonstrates that although IL26 is largely a T17-associated cytokine, it promotes BBB integrity in vitro and in vivo, and using it as a therapy induces amelioration of neuroinflammation.
Introduction: T helper 17 (Th17) cells are CD4+ effector T cells that play an instrumental role in driving pro-inflammation IL-17 production in several autoimmune conditions, such as ankylosing spondylitis (AS).

Aim: Our aim is to identify epigenetic pathways and mechanisms that inhibit Th17 pro-inflammatory cell function. To this end, we use chemical and shRNA knockdown tools to study “readers, writers and erasers of the histone code” that regulate gene transcription.

Results: A focused epigenetic compound screen, comprising 60+ selective epigenetic inhibitors, identified Jumonji-type histone demethylases as playing a fundamental role in regulating Th17 cell inflammatory function. Specifically, using flow cytometry, we showed that inhibition of the JMJD3/UTX demethylases leads to suppression of IL-17 cytokine levels. Transcriptomic analysis revealed a pronounced upregulation of the ATF4 family of transcription factors. We also observed reduced proliferation, which was driven by the ATF4 metabolic stress response, in both healthy donors and patients with AS. Chemical inhibitors of JMJD3/UTX demethylases were overtly impactful on the cellular biochemistry of Th17 differentiated T-cells, with prominent effects on differentiation-related changes in glucose utilization, anaerobic processes, and remarkable changes in amino acid and TCA cycle metabolism. Using Chip-seq and ATAC-seq, we found that this phenotype was driven through global increases in the repressive H3K27me3 histone mark.

Conclusions: We show that inhibition of JMJD3/UTX histone demethylases in Th17 cells leads to an ATF4 metabolic stress response, induction of cellular anergy and a reduction in pro-inflammatory function. Therefore, JMJD3/UTX inhibition may be a tractable therapeutic target in autoimmunity.

WS.C2.03.04
Immunoglobulin A activated myeloid cells induce pathological osteoclast activation in rheumatoid arthritis patients
M. M. J. van Gool, A. J. Breedveld, R. E. Mebius, M. van Endmon;
VU University Medical Center, Amsterdam, Netherlands.

Immunoglobulin A (IgA) is crucial for maintaining homeostasis at mucosal sites. However, it is becoming clear that excessive activation of myeloid cells by IgA via FcαRIi leads to severe inflammation and tissue damage. Recently, our lab has shown that neutrophils get activated by IgA rheumatoid factor (RF) present in the serum and synovial fluid of rheumatoid arthritis (RA) patients. Since IgA RF is correlated with severe bone erosions in RA patients, we hypothesize that IgA contributes to the pathology of RA. Here we show that cellular functions and cytokine release by IgA activated neutrophils and monocytes results in a specific and profound pro-inflammatory response. Only IgA induces migration of neutrophils, followed by monocytes. Moreover, the release of TNF-α by both cells, IL-6 for monocytes and IL-8 for neutrophils was only seen after IgA activation. To determine the effect of IgA activated cells on osteoclast activation, we performed a bone resorption assay in which osteoclasts were cultured in the presence of supernatant of IgA or IgG activated neutrophils and monocytes. Bone resorption was significantly increased when osteoclasts were cultured in the presence of supernatant of IgA activated cells compared to IgG. This suggests that myeloid cells activated by IgA autoantibody complexes play a role in inducing joint damage in RA patients, hereby increasing disease severity. Moreover, we discovered that differentiated osteoclasts also express FcαRI in contrast to Fcγ receptors, therefore osteoclasts can be directly activated by IgA complexes. Blocking FcαRI may represent a novel therapeutic strategy for the treatment of RA.

WS.C2.03.05
Anti-Ly9 (CD229) antibody treatment reduces marginal zone B cell numbers and salivary gland inflammation in a mouse model of Sjögren’s Syndrome
Universitat de Barcelona, Barcelona, Spain.

Sjögren’s Syndrome (SS) is one of the most common chronic autoimmune rheumatic diseases. Characterized by B cell activation, lymphocyte cell infiltration and tissue damage of exocrine glands, it can also present life-threatening extraglandular affections, such as respiratory/hepatic dysfunction, chronic infections and marginal zone B-cell lymphoma. Several biologic agents have been tested in SS but none has shown significant efficacy. Here we report the effects of antibodies against Ly9 (CD229), which is a cell surface molecule that belongs to the SLAM family of immunomodulatory receptors, using NODh2 h4 knockout mice as a model of SS-like disease. Female mice were treated with anti-Ly9 antibody or isotype control at week 24, when all mice present SS-related autoantibodies, salivary gland infiltrates and marginal zone (MZ) B cell pool enlargement. Plasma was collected before and after treatment, and quantified by ELISA and immunofluorescence. B and T lymphocyte subsets from lymphoid tissues and salivary glands were analyzed by flow cytometry. Salivary glands were also studied in paraffin-embedded sections. Autoantibody levels (anti-ANA, anti-Ro, anti-dsDNA and RF) were decreased or impeded to increase over time after anti-Ly9 treatment. Moreover, this treatment induced the depletion of key lymphocyte subsets involved in SS pathology such as MZ and germinal center B cells in the spleen, draining lymph nodes and salivary glands. Importantly, mice receiving anti-Ly9 mAb showed a reduction of the infiltrate within salivary glands (isotype control: 0.61 ±mm² versus anti-Ly9: 0.15 ±mm²). These data indicate that Ly9 is a potential therapeutic target for the treatment of SS.

WS.C2.03.06
Dysregulated expression of RasGRP1 in T cells leads to autoimmunity
T. Daum2, D. Simeonov2, D. Myers2, M. Boers3, G. Mijnheer3, F. van Wijk1, A. Larson3, J. Roseo3, Y. Vercoulen3, M. Feldmann4, T. A. Chatila5, M. M. O. Li6, M. O. Li1, U. Oppermann7, K. Chen1, A. Ozen1, M. D. J.1, T. A. Chaltit3, R. K. Prinjha7, M. Feldmann8, P. Bowness1, Epinova Discovery Performance Unit, GSK, Stevenage, United Kingdom, 1Kennedy institute of Rheumatology, Oxford, United Kingdom, 2Botnar Research Centre, Oxford, United Kingdom, 3Epinova Discovery Performance Unit, GSK, Stevenage, United Kingdom, 4Kennedy institute of Rheumatology, Oxford, United Kingdom.

RasGRP1 is a Ras guanine nucleotide exchange factor (GEF), and an essential regulator of lymphocyte receptor signaling. Aberrant expression of RasGRP1 results in defective positive thymocyte selection in mice. Furthermore, recent case reports describe RasGRP1 deficient patients that suffer from recurrent infections and autoimmunity. It is unclear how RasGRP1 levels are regulated and how aberrant expression contributes to autoimmunity.

Results: Increased expression of RasGRP1 directly results in increased Ras-MAPK signaling in lymphocytes. We detected antinuclear antibodies in the serum of rasgrpl1+ and rasgrpl1-1 mice, suggesting that loss of the SLAM family of immunomodulatory receptors, such as NODh2 h4, is a major factor in the development of autoimmunity. In patients with juvenile idiopathic arthritis, we decreased the expression levels of rasgrpl1 in CD4+ T cells isolated from synovial fluid compared to peripheral blood. Next, we analyzed H3K27 acetylation profiles and identified 2 enhancer regions of the RasGRP1 gene.

Conclusions: We identified an enhancer region upstream of rasgrpl1 that is required for regulation of rasgrpl1 expression, and SNPs in this region are associated with autoimmunity. Furthermore, our data reveal that decreased expression of RasGRP1 results in autoimmunity in mice, and that rasgrpl1 expression is decreased in T cells in the synovial fluid of patients with juvenile idiopathic arthritis. Together, we show that proper regulation of RasGRP1 expression is essential to prevent inflammatory disease.

WS.C2.04.01
Functional reprogramming of regulatory T cells in the absence of FoxP3
L. M. Charbonnier1, Y. Cui1, D. Lopez1, J. J. Blessing2, M. I. Garcia-Lorente3, K. Chen4, A. Ozen5, M. D. J.1, T. A. Chaltit6, 1Boston Children’s Hospital and Harvard Medical School, Boston, United States, 2University of California, Los Angeles, United States, 3Cincinnati Children’s Hospital Medical Center, Cincinnati, United States, 4David Geffen School of Medicine at the University of California Los Angeles, Los Angeles, United States, 5University of Utah School of Medicine, Salt Lake City, United States, 6Marmara University Faculty of Medicine, Istanbul, Turkey.

Foxp3-deficient regulatory T (Treg) cells lack suppressor function and manifest a T effector (Teff) cell-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant Treg cells lack suppressor function and manifest a Teff-like phenotype.

Conclusions: We identified an enhancer region upstream of rasgrpl1 that is required for regulation of rasgrpl1 expression, and SNPs in this region are associated with autoimmunity. Furthermore, our data reveal that decreased expression of RasGRP1 results in autoimmunity in mice, and that rasgrpl1 expression is decreased in T cells in the synovial fluid of patients with juvenile idiopathic arthritis. Together, we show that proper regulation of RasGRP1 expression is essential to prevent inflammatory disease.

WS.C2.04.02
Therapy of autoimmunity disorders

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1Amsterdam Rheumatology & Immunology Center | Department of Experimental Immunology, Academic Medical Center/University of Amsterdam, Amsterdam, Netherlands, 2Department of Experimental Immunology, Academic Medical Center/University of Amsterdam, Amsterdam, Netherlands, 3Respiratory, Inflammation and Autoimmunity, AMED Biotech Unit, AstraZeneca, Gothenburg, Sweden.

Immune response profiling reveals signaling networks mediating TNF-blocker function and predictors of therapeutic responses in spondyloarthritis patients.

We found that anti-TNF therapy induces specific changes in immune responses of patients to various stimuli. These changes can be measured in stimulated, but not resting immune cells and are detectable already after a single injection of anti-TNF. Quantitative set analysis for gene expression (QlSAGE) of the stimulation cultures revealed that the gene modules most affected by anti-TNF therapy are NF-kB transcription factors and target genes, suggesting that TNF-blockers primarily act by breaking TNF- and IL-1-dependent feed-forward loop of NF-kB activation. We also tested if induced immune responses correlate with therapeutic responses. Using machine-learning algorithms, we found that expression of several molecules regulating key steps of leucocyte migration and invasiveness was significantly higher in patients responding to anti-TNF therapy following LPS-stimulation, while expression of cytokotic and T-cell genes was higher in non-responders. The random forest model that we trained and validated using 13 selected biomarkers has a predictive power of 0.8. We propose that immune response profiling of patients before therapy is a powerful new strategy to help guiding clinical decisions.

Targeting NF-κB signalling in B cells: a potential new treatment modality for antibody mediated autoimmune diseases

B cell proliferation. Co-engaging CD19xCD47 prevented CD19 clustering and its migration to the BCR. The CD19 mAb showed no impact on either CD19 clustering or its migration to the BCR. The CD47xCD19 BsAb targeted in auto-immune diseases and B cell malignancies, is altered in the presence of the BsAb.

Materials and Methods: Using flow cytometry-based assays, we investigated the function of the BsAb in B cell homeostasis. Treatment of the transformed SCID mice either with DNA-like chimeric and anti-Anx A1 antibody prevented appearance of anti-DNA antibodies and proteinuria, while the PBS-injected animals had high levels after the transfer. The treatment reduced the levels of disease-associated cytokines also. Conclusions: It is possible to down-regulate the activity of pathogenic human T and B cells in humanized SLE-SCID mouse model of SLE by targeting Anx A1 or CR1 with a specific monochimeric or bi-specific antibody.

Suppression of autoreactive T and B lymphocytes by selective therapy in humanized murine SCID model of systemic lupus erythematosus

Introduction: Specific-self B and T cells play a main role in pathogenesis of Systemic lupus erythematosus (SLE) and are a logical target for selective therapy. The complement receptor type 1 (CR1) on human B-lymphocytes has suppressive activity and engagement of this receptor inhibits B cell activation. The protein Annexin A1 (Anx A1), is a modulator of the immune system and abnormal expression was found on activated B and T cells during human autoimmunity. We hypothesize that it may be possible to down-modulate the activity of autoreactive T and B cells from SLE patients in humanized SCID mouse model by treating them with a neutralizing antibody against Anx A1 or by protein engineered molecules, which co-crosslink the BCR and CR1.

Materials and Methods: Protein chimeric molecules construction, Immunodeficient SCID mice transfer with human PBMC from SLE patients, ELISA for dsDNA antibodies and cytokines, flow cytometry for apoptosis and activation markers, ELSpot and MTT assays, protein array. Results: Reconstituted SCID mice showed presence of several auto-antibodies, as well as immunoglobulin deposition in the renal glomeruli. Treatment of the transformed SCID mice either with DNA-like chimeric and anti-Anx A1 antibody prevented appearance of anti-DNA antibodies and proteinuria, while the PBS-injected animals had high levels after the transfer. The treatment reduced the levels of disease-associated cytokines also. Conclusions: It is possible to down-regulate the activity of pathogenic human T and B cells in humanized SLE-SCID mouse model of SLE by targeting Anx A1 or CR1 with a specific monochimeric or bi-specific antibody.

Interleukin-6 Receptor inhibition, as first-line b-DMARD, affects B cell subpopulations distribution through epigenetic modifications in Rheumatoid Arthritis patients.

C. Di Mario, B. Tolusso, S. Aliverninib, A. Fedele, L. Petricca, M. Gigante, G. Ferraccioli, E. Gremese; 
1Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 2University Hospital “St. Ivan Rilski”, Sofia, Bulgaria, 3Immunoology Research Group, Hungarian Academy of Sciences, Budapest, Hungary.

Introduction: B cell maturation is controlled by microRNA-155(miR-155) and IL-6. IL-6 activity in the joint is a component of the inflamed damage. Biochemical evidence suggests that overexpression of miR-155 in RA leads to an increase of the expression of the pathogenic activity of B cells.

Materials and Methods: We investigated if IL-6R inhibitor acts restoring CD19 expression in PB-derived CD19 cells after 3-6-12-18months follow-up peripheral blood(PB)-derived CD19 cells were isolated by magnetic microbeads(Miltenyi) and miR-155 and PU.1 endogenous expression was determined by RT-PCR. Moreover, B cells subpopulations were assessed through FACS analysis (lgD/CD27 classification). IL-6 plasma levels was assessed by ELISA. ACE/ELURAL criteria were used to assess the response rate of PB-derived CD19 cells of healthy individuals(HC) were used as comparison. Results:At study entry, RA achieved a significant difference of lgD/CD27 CD19 cells percentage compared to not responding patients(p<0.05). At baseline, PB-derived CD19 cells of RA showed significantly higher endogenous expression of miR-155(p<0.04) than HC. Moreover, RT-PCR showed that IL-6R inhibition significantly reduces endogenous miR-155 expression in PB-derived RA CD19 cells after 3 months(p<0.05) restoring PU.1 expression in PB-derived CD19 cells after 6 months(p<0.05) only in RA achieving disease remission. Conclusions:IL-6R inhibitor acts restoring CD19 cells homeostasis through epigenetic modulation in RA repressing endogenous expression of miR-155 in PB-derived CD19 cells and restoring PU.1 expression mimicking the decrease of lgD/CD27 CD19 cells rate in RA achieving disease remission.

Co-engaging CD47 and CD19 with a bispecific antibody mimics B cell receptor cross-linking

1Novimmune SA, Geneva, Switzerland, 2INSERM, Lyon, France, 3Flow cytometry core facility, Geneva, Switzerland.

Introduction: A CD47xCD19 bispecific antibody (BsAb) is being developed for the treatment of B cell malignancies. We investigated if B cell receptor (BCR) signaling, a key pathway targeted in autoimmune diseases and B cell malignancies, is altered in the presence of the BsAb.

Materials and Methods: Using flow cytometry-based assays, we investigated whether co-engagement of CD19xCD47 could inhibit BCR cross-linking-induced B cell proliferation and alter cell surface dynamics of BCR/CD19. Western blot and gene array were used to investigate the downstream signaling pathways. Results: We confirmed the role of CD19 in regulating BCR signaling by showing that bispecific targeting of CD19 with a monoclonal antibody (mAb) inhibited BCR-induced cell proliferation. In contrast, monovalent engagement of CD19 failed to abrogate B cell proliferation. Interestingly, the CD47xCD19 BsAb inhibited B cell proliferation more potently than the BsAb. The inhibitory effect of the BsAb was not attributable to CD47 binding alone as the anti-CD47 mAb had no effect on B cell proliferation. Co-engaging CD19xCD47 prevented CD19 clustering and its migration to the BCR. The CD19 mAb showed no impact on either CD19 clustering or its migration to the BCR. The reduced CD19 migration to the BCR resulted in a decreased CD19 phosphorylation upon BCR cross-linking. Gene array analysis confirmed that the BsAb and the anti-CD19 mAb employed different mechanisms in controlling B cell activation. Conclusions: These data suggest that the BsAb may provide therapeutic benefit in settings of autoimmunity and B cell malignancies by dampening BCR-stimulated B cell proliferation.
The presence of pretransplant donor-specific anti-HLA antibodies (DSA) is associated with increased risk of kidney graft failure. HLA antibody detection by single-antigen bead assay (SAB) is much more sensitive than by complement-dependent crossmatches (CDC-XM). Here, we studied the impact of SAB-detected DSA on graft survival for all CDC-XM negative kidney transplants performed between 1995 and 2006 in the Netherlands. The impact was most pronounced in the 3237 deceased-donor transplantations: transplantations positive for SAB-detected DSA (N=430/3237) had a 16% worse 10-year graft survival than those without DSA. Due to the lack of second-field donor HLA typing, donor-specificity of the SAB-detected antibodies was initially determined at serological (split) level. The SAB assay however allows for second-field definition of antibody-specificity. Using the HLA-epitope registry (http://www.epiregistry.com.br) the most likely epitope-specificity of the detected antibodies was defined. NMDP-HLA-haplotype frequencies were used to determine the most likely second-field HLA types of all recipients and donors.

Combination of these tools enabled the determination of donor anti-HLA-epitope specific antibodies (DESA). Pretransplant DESA-positive deceased-donor transplantations (N=312/3237) had a 20% poorer 10-year graft survival than those without DESA. A higher number of DESA led to an even worse graft survival: transplantations with more than 2 DESA (N=236) had a 25% poorer graft survival compared to transplantations without DESA. We conclude that even without the exact knowledge of both the HLA-epitope specificity of the SAB-detected antibodies and the mismatched donor HLA-epitopes, the number of pretransplant DESA might be a better parameter to stratify risk than the presence of serologically-defined DSA.

**Results:** Remarkably, no relation was found between donor-specific IFN-γ pc frequency and rejection in both groups. However, significantly higher donor-specific IL-21 pc numbers were found in patients who developed rejection compared to those without rejection in both the late (p=0.0008) and early rejection group. ROC-curve analysis of donor-specific IL-21 pc frequencies distinguished the development of rejection from non-rejection with a specificity of 88% and 80% in the late and early rejection group, and a sensitivity of 50% and 73%, respectively. Patients with low IL-21 pc frequencies had a significantly increased rejection free survival rate in both the late rejection group (p=0.0008) and early rejection group (p=0.0005) compared to those with high frequencies.

**Conclusions:** The data indicate a high degree of potential mismatch in NK cell diversity between donor and recipient in the case of solid organ transplantation. Further analyses will be performed in order to evaluate the functional consequences and clinical relevance of these antibodies in transplantation. Supported by DFG grant SF873B/AS 1/1.

**WS.C3.03.01 Transplantation - pathogenesis and early diagnosis**

**WS.C3.01.01** Improved risk stratification of pretransplant donor-specific antibodies with epitope analyses

**E. G. Kamburova**, on behalf of the PROCARE consortium, H. G. Otten; University Medical Center Utrecht, Utrecht, Netherlands.

The major histocompatibility complex (MHC) contains the most polymorphic genetic system in humans, the human leukocyte antigen (HLA) genes of the adaptive immune system. High allelic diversity in HLA is argued to be maintained by balancing selection, such as negative frequency-dependent selection or heterozygote advantage. Selective pressure against immune escape by pathogens can maintain appreciable frequencies of many different HLA alleles. The selection pressures operating on combinations of HLA alleles across loci, or haplotypes, have not been extensively evaluated since the high HLA polymorphism necessitates very large sample sizes, which have not been available until recently. We aimed to evaluate the effect of selection operating at the HLA haplotype level by analyzing 5 locus haplotype frequencies derived from over six million individuals genotyped by the NMDP registry.

In contrast with alleles, HLA haplotype diversity patterns suggest purifying selection, as certain HLA allele combinations co-occur in high linkage disequilibrium. Linkage disequilibrium is positive (D>0) among frequent haplotypes and negative (D<0) among rare haplotypes. Fitting the haplotype frequency distribution to several population dynamics models, we found that the best fit was obtained when significant positive frequency-dependent selection (FDS) was incorporated. Finally, the Ewens-Watterson test of homozygosity showed excess homozygosity for 5-locus haplotypes within 23 US populations studied.

Haplotype diversity is most consistent with purifying selection for HLA Class I, and was not inferred for HLA Class II. We discuss our empirical results in the context of evolutionary theory, exploring potential mechanisms of selection that maintain high linkage disequilibrium in MHC haplotype blocks.

**WS.C3.01.02** Detection of alloantibodies against NK cell antigens after transplantation

**T. Langer Jacobus, R. E. Schmidt, C. S. Falk, E. Jackel, F. W. Vondran, C. Ferreira de Figueiredo, R. Jacobs; MHZ, Hannover, Germany.**

Introduction: Both IFN-γ and IL-21 support induction and expansion of highly-reactive cytotoxic CD8 T-cells. In addition, IL-21 is a key cytokine for differentiation of alloantigen activated naive and memory B-cells into antibody-producing plasma cells. We questioned whether the donor-specific IFN-γ and IL-21 producing cells (pc) frequency can predict kidney transplant rejection.

Methods: PBMC samples from 47 patients obtained at 6 months after kidney transplantation of whom 14 patients developed a late rejection (>6 months). In addition, pre-transplantation samples of 38 patients of whom 17 patients had an early rejection (<3 months). The frequency of donor-reactive circulating IFN-γ and IL-21 pc was determined by ELISPOT assay.

Results: Remarkably, no relation was found between donor-specific IFN-γ pc frequency and rejection in both groups. However, significantly higher donor-specific IL-21 pc numbers were found in patients who developed rejection compared to those without rejection in both the late (p=0.020) and early (p=0.024) rejection group. ROC-curve analysis of donor-specific IL-21 pc frequencies distinguished the development of rejection from non-rejection with a specificity of 88% and 80% in the late and early rejection group, and a sensitivity of 50% and 73%, respectively. Patients with low IL-21 pc frequencies had a significantly increased rejection free survival rate in both the late (p=0.0008) and early rejection group (p=0.0005) compared to those with high frequencies.

Conclusion: The frequency of donor-specific IL-21 producing cells is linked to an increased risk of rejection, giving it the potential to be a new biomarker in predicting rejection in different phases of transplantation.

**WS.C3.01.03** HLA Class I Haplotype Diversity Is Consistent with Selection for Frequent Existing Haplotypes

**Y. Louzoun,1 I. Alteri,2 M. Maires2, L. Gragert3;1 Bar Ilan, Ramat Gan, Israel, CIBMTR, Minneapolis, United States, 2Tulane University, New Orleans, United States.**

High allelic diversity in HLA is argued to be maintained by balancing selection, such as negative frequency-dependent selection or heterozygote advantage. Selective pressure against immune escape by pathogens can maintain appreciable frequencies of many different HLA alleles. The selection pressures operating on combinations of HLA alleles across loci, or haplotypes, have not been extensively evaluated since the high HLA polymorphism necessitates very large sample sizes, which have not been available until recently. We aimed to evaluate the effect of selection operating at the HLA haplotype level by analyzing 5 locus haplotype frequencies derived from over six million individuals genotyped by the NMDP registry.

In contrast with alleles, HLA haplotype diversity patterns suggest purifying selection, as certain HLA allele combinations co-occur in high linkage disequilibrium. Linkage disequilibrium is positive (D>0) among frequent haplotypes and negative (D<0) among rare haplotypes. Fitting the haplotype frequency distribution to several population dynamics models, we found that the best fit was obtained when significant positive frequency-dependent selection (FDS) was incorporated. Finally, the Ewens-Watterson test of homozygosity showed excess homozygosity for 5-locus haplotypes within 23 US populations studied.

Haplotype diversity is most consistent with purifying selection for HLA Class I, and was not inferred for HLA Class II. We discuss our empirical results in the context of evolutionary theory, exploring potential mechanisms of selection that maintain high linkage disequilibrium in MHC haplotype blocks.

**WS.C3.01.04** High numbers of donor-specific IL-21 producing cells predict rejection after kidney transplantation

**N. M. van Besouw,1 L. Van,1 R. de Kuiper,1 M. Klepper,1 D. Reijerkerk,1 D. L. Raelen,1 F. H. Claas1, M. C. Claesen-van Groningen,1 D. A. Hesselink1, C. C. Baan1;2 Erasmus MC, Rotterdam, Netherlands, 2Trans West China Hospital, Chengdu, China, 3LUMC, Leiden, Netherlands.**

Introduction: Both IFN-γ and IL-21 support induction and expansion of highly-reactive cytotoxic CD8 T-cells. In addition, IL-21 is a key cytokine for differentiation of alloantigen activated naive and memory B-cells into antibody-producing plasma cells. We questioned whether the donor-specific IFN-γ and IL-21 producing cells (pc) frequency can predict kidney transplant rejection.

Methods: PBMC samples from 47 patients obtained at 6 months after kidney transplantation of whom 14 patients developed a late rejection (>6 months). In addition, pre-transplantation samples of 38 patients of whom 17 patients had an early rejection (<3 months). The frequency of donor-reactive circulating IFN-γ and IL-21 pc was determined by ELISPOT assay.

Results: Remarkably, no relation was found between donor-specific IFN-γ pc frequency and rejection in both groups. However, significantly higher donor-specific IL-21 pc numbers were found in patients who developed rejection compared to those without rejection in both the late (p=0.020) and early (p=0.024) rejection group. ROC-curve analysis of donor-specific IL-21 pc frequencies distinguished the development of rejection from non-rejection with a specificity of 88% and 80% in the late and early rejection group, and a sensitivity of 50% and 73%, respectively. Patients with low IL-21 pc frequencies had a significantly increased rejection free survival rate in both the late (p=0.0008) and early rejection group (p=0.0005) compared to those with high frequencies.

Conclusion: The frequency of donor-specific IL-21 producing cells is linked to an increased risk of rejection, giving it the potential to be a new biomarker in predicting rejection in different phases of transplantation.
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WORKSHOPS

WS.C3.01.05 Immunization against the polysaccharide Poly-N-acetylgalactosamine reduces graft-versus-host disease without affecting microbial diversity
J. Hüldenkrüger,1,2 O. S. Thomas,3,4 Z. Gao,5 S. Duquevesse,5 S. Kirschnek,6 A. Schmitt-Gräff,6 M. J. Blaser,6 G. B. Pier,7 R. Zeiser1
1Department of Hematology, Oncology and Stem Cell Transplantation, Freiburg, Germany, 2Sperman Graduate School of Biology and Medicine (SGSBM), University Freiburg, Germany, 3Faculty of Biology, University of Freiburg, Germany, 4Department of Medicine, New York University Langone Medical Center, New York, United States, 5Institute of Medical Microbiology and Hygiene, University Medical Center Freiburg, Freiburg, Germany, 6Institute of Surgical Pathology, Faculty of Medicine, Freiburg, Germany, 7Division of Infectious Diseases, Department of Medicine, Brigham and Women’s Hospital/Harvard Medical School, Boston, United States.

Acute graft-versus-host disease (aGVHD) is a severe complication after allogeneic hematopoietic cell transplantation (allo-HCT). Opportunistic infections, especially during the period of neutropenia, are frequently occurring in allo-HCT patients and demand antibiotic intervention to prevent bacteremia and sepsis. However antibiotics lead to a loss of microfloral diversity which is connected to a higher incidence of aGVHD. Antibacterial therapies that eliminate invading bacteria without impacting the diversity of the microflora are therefore highly desirable. A potential solution offer anti-bacterial antibodies that target pathogens ultimately leading to elimination by innate immune cells.

We investigated the potency of active and passive immunization against the polysaccharide Poly-N-acetylgalactosamine (PNGA) that is expressed on numerous pathogens in a mouse model of aGVHD. Anti-PNGA antibody treatment in the early phase after allo-HCT reduced aGVHD-related mortality and histopathological aGVHD severity. The treatment did not disturb the intestinal microbial composition as determined by 16s rDNA sequencing. Mechanistically we could show that neutrophils were recruited more abundantly to the intestinal tract following anti-PNGA treatment but exhibited a reduced activation profile as determined by their myeloperoxidase activity. Vaccination against PNGA prior to allo-HCT induced high antibody titers in mice that were detectable throughout the early phase of aGVHD and significantly reduced aGVHD-related mortality.

In summary, antibody-treatment-based and vaccination-based anti-PNGA immunization strategies open a new strategy to interfere with aGVHD without affecting microbial diversity.

WS.C3.01.06 Motifs of peptides binding in HLA-DP and their relation with T-cell epitope groups relevant in stem cell transplantation
P. Van Balen1, M. G. Kester1, M. de Kleer1, P. Crivello1, A. H. De Ru1, M. Van de Meent1, I. Jedema2, M. H. Heemskerk1, K. Fleischauer1, J. H. Falkenburg3, P. A. Van Veelen2
1Leiden University Medical Center, Leiden, Netherlands, 2Institute for Experimental Cellular Therapy, Essen, Germany.

Introduction
Permissiveness of HLA-DP mismatches in allogeneic stem cell transplantations is based on categorisation of HLA-DP alleles into T-cell epitope (TCE) groups. TCE1 and TCE2 are clearly defined, but TCE3 represents a relatively heterogeneous group. To investigate whether characterization of peptides binding in HLA-DP can be of use to re-define TCE groups, we analyzed the peptide profiles of 11 HLA-DP molecules. Methods To investigate peptides presented in HLA-DP encoded by DPB1*09:01, 10:01, 17:01 (TCE1), DPB1*03:01, 14:01 (TCE2) and DPB1*01:01, 02:01, 04:01, 04:02, 05:01, 13:01 (TCE3), HLA-DPB1 typed EBV-LCL were expanded. HLA-DP immunofluorimetry chromatography using anti-HLA-DP B7.21 antibody was performed, followed by analysis of eluted peptides using mass spectrometry and subsequent alignment and clustering using Gibbs sampling to obtain motifs of peptides binding in different HLA-DP molecules. Results All alleles from TCE1 had a similar KAL motif at P1, P6, and P9, reflecting their structural similarity in the relevant hypervariable region. This motif was shared also by DPB1*14:01 from TCE2, while the motif for HLA-DP81*03:01 was different (RAS). TCE2 alleles could be classified into two main groups: those with a totally different FFV motif at P1, P6, and P9 (DPB1*13:01, 04:01, 04:02 with high structural similarity), and those with a motif more similar to TCE1 in P1, but different in P6 and P9 (KXX), (DPB1*01:01, 05:01 and 13:01). Conclusion The current categorization into TCE groups may need to be adjusted based on these results, especially with regard to potentially permissive or non-permissive mismatches within HLA-DP alleles in TCE group 3.

WS.C3.02 T regulatory cells derived and other cellular therapies in transplantation

WS.C3.02.01 Development of a novel protocol to produce massive amounts of autologous thymus-derived Treg cells (thyTreg) to be employed as cellular therapy in transplanted children. A new paradigm in the treatment of immunological diseases
E. Bernaldo de Quirós1, V. Pérez1, M. Clemente1, J. Gil-Jaurena1, M. Fernández-Santos1, S. Sudrez1, V. Plosencia1, A. Acosta1, M. Camino1, N. Gill2, E. Panadero3, C. Medrano3, M. Pion1, R. Correa-Rocha1
1Institute for Experimental Cellular Therapy, Essen, Germany, 2Department of Medicine, Brigham and Women’s Hospital/Harvard Medical School, Boston, United States, 3Cell Culture Unit, Gregorio Marañón Health Research Institute, Madrid, Spain.

Background: to investigate the feasibility of developing a new GMP compatible protocol for the production of autologous thyTreg cells in children undergoing bone marrow transplantation (BMT). Methods: a pilot study was performed. A protocol was developed to isolate thyTreg cells from peripheral blood (PB) and thymus tissue (T) of children undergoing BMT. Results: The thyTreg product manufactured in the GMP facility have been confirmed. After receiving the approval from the Spanish Drug Agency (AEMPS), we will initiate the first clinical trial worldwide (phase I/IIa) to evaluate the safety and feasibility of employing autologous thyTreg in children undergoing transplantation. Conclusions: Massive amounts of pure, highly suppressive Thy-Treg obtained with our novel GMP-compatible protocol are suitable for use as cellular immunotherapy to prevent graft-versus-host reaction in transplant children. The clinical use of these Thy-Treg could increase the graft survival in transplanted patients, and may revolutionize the treatment of other immunological diseases.

WS.C3.02.02 Effective Treg therapy of lethal acute graft-versus-host disease
C. Riege1, T. Böld1, E. Huber1, P. Hoffmann1, M. Edinger1
1University Hospital Regensburg, Regensburg, Germany, 2Regensburg Center for Interventional Immunology (RCI), University Regensburg, Regensburg, Germany.

Allogeneic stem cell transplantation (SCT) is the treatment of choice for many hematologic diseases, carrying the inherent risk of acute graft-versus-host disease (aGVHD) that is caused by alloreactive donor T cells and mainly targets the gastrointestinal (GI) tract, skin and liver. We previously showed that co-transplantation of donor CD4+CD25+ regulatory T cells (Treg) prevents lethal aGVHD even in complete MHC-mismatched SCT models, a strategy already confirmed in first clinical trials. Here, we examined the efficacy of donor Treg to treat established aGVHD. We isolated and in vitro expanded CD4+CD25+CD62L+ Treg for 14d from BALB/c donors that multiplied up to 80-fold and upregulated GI-tract homing receptors in vitro. Upon transfer into CB6F1 mice with established aGVHD (d15 post SCT) they rescued >65% of recipients from otherwise lethal aGVHD and still 30% if administered in very late disease stages (d63 post SCT). Initially, therapeutically administered Treg (taTreg) migrated predominantly into the gastrointestinal tract. Here, they reduced the influx of neutrophils and conventional T cells into the colon and diminished their proliferation and secretion of pro-inflammatory cytokines. taTreg persisted long-term, maintained their phenotype and Foxp3 expression in GVHD and non-GVHD target organs and still represented 5-25% of the local Treg population by d100 post SCT. They promoted tissue regeneration in the GI tract, as indicated by the reappearance of Paneth cells, and blocked further lymphoid tissue destruction thereby fostering immune reconstitution. Summarized, our results demonstrate that donor Treg transfer seems effective for the treatment of severe aGVHD. Supported by DFG.
WS.C3.02.03  Regression of Treg specificity toward allograft transplants to sustainably prevent rejection

R. Alhamawi1, C. Desmytere2, R. Albuquerque1, S. Lee1, N. D. Jones1;

1Institute of immunology and immunotherapy, Birmingham, United Kingdom, 2Medical laboratory technology department, Madinah, Saudi Arabia.

Foxp3+ regulatory T cells (Tregs) are with a potent immunosuppressive capacity that may be used as a cellular therapy to suppress autoimmunity and transplant rejection. It has been suggested that redirecting the specificity of Tregs to allograft might increase their potency to suppress rejection compared to polyclonal cells. With this in mind, we have optimised a protocol to expand mouse Tregs in vitro using anti-CD3/CD28 beads in the presence IL-2 and TGFβ to facilitate genetic manipulation. This protocol allowed a 40-fold to 6-fold expansion of Tregs with limited contamination with conventional T cells. In an assay of non-specific suppression where both conventional T cells (Tcons) and Tregs were activated by anti-CD3/anti-CD28 mAbs, expanded Tregs were found to suppress the conventional T cell response at least as well as freshly isolated Tregs. Importantly, the expanded Tregs proved to be amenable to retroviral-transduction with a plasmid carrying T cell receptor (TCR) and a β chains that confer reactivity to the MHC class I allograft H2K. Analysis of transduced Tregs revealed that 85±5% expressed the alloreactive TCR following transduction. Finally, the TCR gene-engineered Tregs were tested for their ability to suppress an allogeneic CD4+ and CD8+ T cell response in vitro. We found that the TCR engineered Tregs suppressed alloreactive T cell responses with increased potency compared to expanded mock-transduced Tregs in vitro. Taken together these data suggest that indeed redirecting Tregs to transplant antigens may improve the efficacy of these cells when used as a cellular therapy to prevent transplant rejection.

WS.C3.02.04  Extracellular adenosine reversibly inhibits the activation of human regulatory T cells and negatively influences the achievement of the operational tolerance in liver transplantation

A. Baraja-Mazo1, B. Revilla-Nuñez1, L. Martinez-Alarcón1, J. Herrero1, A. El-Tayeb1, C. Müller2, P. Aparicio1, P. Pelegrín1, J. Pons1;

1Department of Liver Transplantation and Immunology, Instituto de Ciencias de la Salud, Hospital Universitario Miguel Servet, Zaragoza, Spain, 2Laboratory of Molecular Biology, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico.

The possibility of stimulating or inducing a state of operational tolerance in transplantation is gaining strength. In murine models, a differential of extracellular adenosine (eADo) for regulatory (Tregs) and effector (Teffs) T cells has been proposed: inhibiting Teffs activity and inducing Tregs. The aim of the present study was to analyze the extracellular nucleotides in human T cells, and moreover, examine the influence of CD39 and CD73 ectonucleotidasases and subsequent adenosine signaling through adenosine 2A (A2A) and 3 (A3) receptors on the induction of clinical tolerance after liver transplantation. The action of extracellular nucleotides in human T cells was analyzed by in vitro experiments with isolated T cells. Additionally, 17 liver transplant (LT) patients were enrolled in an immunosuppression withdrawal trial, and the differences in the CD39-CD73-A2A_A3 axes were compared to tolerant and non-tolerant patients. In contrast to the mouse model, the activation of human Tregs was inhibited in a similar rate to T effs in the presence of eADo. The inhibitory response was reversible. Moreover, the relative expression of the enzyme responsible for irreversible degradation of ADo, adenosine deaminase (ADA) was much higher in tolerant patients with respect to the non-tolerant group along the immunosuppression withdrawal process. Our data support the idea that extracellular adenosine signaling and its degradation by ADA may play a role in the complex system of regulation of liver transplantation tolerance. In addition, we have the capacity of ADA expression to differentiate the operational tolerance state at the first steps of the immunosuppression withdrawal process.

WS.C3.02.05  Targeting recipient antigen presenting cells with sialic acid-modified alloantigen to promote transplantation tolerance

M. Sen1, K. Ratnakoshy2, M. Ambrosini2, D. Guiliano1, Y. van Kooyk1, G. Lombardi1, L. A. Smyth2;

1School of Health, Sport and Bioscience, University of East London, London, United Kingdom, 2MRC Centre for Transplantation, King's College London, London, United Kingdom.

Sialic acid binding immunoglobulin-like lectins (siglecs) are inhibitory receptors expressed on dendritic cells (DCs) that bind to sialic acid ligands. These receptors have previously been targeted to murine DCs to induce antigen-specific tolerance in an autoimmune mouse model. To date, it has not been established whether targeting these receptors can induce transplantation tolerance; therefore our aim was to target siglecs-expressing recipient DCs with sialic acid-modified donor alloantigen MHC class I K' peptide (sia- alloantigen). Targeted BMDCs had a reduced capacity to induce antigen specific CD4+ T cell proliferation and effector cytokine production. In addition, co-culturing antigen specific CD4+ T cells with sia- alloantigen treated BMDCs, led to 3-fold percentage increase in CD4+FOXP3+ regulatory T cells (Tregs). Flow cytometry analysis suggests that sia- alloantigen binds to DCs in vitro and in vivo. Targeting sia- alloantigen to DCs in B6.RAG-2/- recipient mice reconstituted with T cells, led to significant skin graft prolongation. To assess which DC subset was involved, B6.BATF3-/- mice, which lack the CD103+ and CD103- DC subsets were compared with B6 mice. Skin transplant prolongation, reduced alloantigen body production and an increased proportion of Tregs were observed only in B6.BATF3-/- mice after treatment with sia- alloantigen. In conclusion, targeting siglec on recipient DCs negative DCs with sia- alloantigen may represent a novel DC targeting regimen to regulate allore cognition.

WS.C3.02.06  Third party virus-specific T cells for treatment of viral reactions in immunocompromised patients and risks of allo-HLA cross-reactivity

W. Husman1, D. A. Lebour1, L. Hagenom1, D. Amess1, F. J. Folkenburg1, J. Jedema1;

1SANGUIN, Amsterdam, Netherlands, 2LUMC, Leiden, Netherlands.

Adoptive transfer of partially HLA-matched virus-specific T cells from healthy third party donors has a potential strategy to temporarily provide anti-viral immunity to immunocompromised patients. However, these T cells harbor the risk of mediating allo-HLA cross-reactivity. To assess the magnitude of this risk, we examined the occurrence and diversity of allo-HLA cross-reactivity mediated by T cell populations targeting different proteins from cytomegalovirus (CMV), Epstein-Bar virus (EBV) and adenovirus (AdV) using an allogeneic EBV/LCL panel covering 116 different HLA molecules and secretion of IFNγ as read-out. Allo-HLA cross-reactivity patterns were confirmed using K562 cells expressing single HLA molecules upon retroviral transduction. A significant proportion of HLA-A*01:01/02:01/07:06 restricted virus-specific T-cell populations (n=170) isolated from 27 healthy donors (30% of the CMV, 46% of the EBV and 36% of the AdV-specific T-cell populations tested) exerted allo-HLA cross-reactivity against one or more allogeneic EBV/LCLs in this analysis. However, for some specificities (e.g. HLA-A*02:01-restricted EBV-BZLF1-RAK) almost all tested populations (n=9/13) showed profound allo-HLA cross-reactivity. Separate analysis of subcultures, sorted on TCR-Vβ usage from the bulk single specificity T cell population, revealed specific allo-HLA cross-reactivity patterns. Furthermore, recurrent patterns of allo-HLA cross-reactivity were observed for virus-specific T cell cultures expressing the same TCR-Vβ family that were isolated from different donors. These data indicate that allo-HLA cross-reactivity by third party virus-specific T cells can be a serious problem and that the magnitude and diversity may be associated with the level of T-cell receptor oligoclonality within a certain T-cell population.

WS.C4.01  Manipulation of tolerogenic pathways

BS.C4.01.01  PD-1 regulates the expression of HELIOS in self-reactive CD8+ and CD4+ T cells

N. Rodriguez Rodriguez1, S. A. Apostolidis2, G. C. Tsokos2, O. Arrieta2, J. C. Crispin2;

1Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico, 2Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico, 3Instituto de Investigaciones Medicas, Harvard Medical School, Boston, United States, 4Instituto Nacional de CanceroLOGIA, Mexico City, Mexico.

Self-reactive CD8+ T lymphocytes that recognize cognate antigen in peripheral tissues become inactivated, downregulate CD8, and express PD-1, becoming PD-1+ T cells. PD-1 regulates the expression of HELIOS in self-reactive CD8+ T cells together with a double negative (DN) T cells, and CD4+CD8+ T cells. In an in vitro assay of non-specific suppression where both conventional T cells (Tcons) and Tregs were activated by anti-CD3/anti-CD28 mAbs, expanded Tregs were found to suppress the conventional T cell response at least as well as freshly isolated Tregs. Importantly, the expanded Tregs proved to be amenable to retroviral-transduction with a plasmid carrying T cell receptor (TCR) and a β chains that confer reactivity to the MHC class I alloantigen H2K. Analysis of transduced Tregs revealed that 85±5% expressed the alloreactive TCR following transduction. Finally, the TCR gene-engineered Tregs were tested for their ability to suppress an allogeneic CD4+ and CD8+ T cell response in vitro. We found that the TCR engineered Tregs suppressed alloreactive T cell responses with increased potency compared to expanded mock-transduced Tregs in vitro. Taken together these data suggest that indeed redirecting Tregs to transplant antigens may improve the efficacy of these cells when used as a cellular therapy to prevent transplant rejection.

In patients with small-cell lung carcinoma that received pembrolizumab, anti-PD-1 administration significantly reduced the frequency of HELIOS+ DN T cells together with a reduction in the frequency of HELIOS+ T cells. RNA-sequence and shRNA studies proved that HELIOS is a central hub that regulates several transcriptional effects downstream of PD-1 signaling required to curb T cell activation. In conclusion, by promoting HELIOS expression, which regulates the PD-1-imposed transcriptional program, PD-1 restrains the expansion of self-reactive CD8+ and DN lymphocytes.
WS.C4.01.02  Immunotherapy with apitopes® blocks the immune response to thyroid stimulating hormone receptor in HLA-DR transgenic mice

E. Schurgers1, F. de Groot2, C. Wraith2, K. Martin2, L. Janss2.

1Apitope International NV, Diepenbeek, Belgium, 2Apitope Technology (Bristol) Ltd, Chepstow, United Kingdom.

Graves’ disease is an endocrine, autoimmune disorder mediated by autoreactive T and B lymphocytes responding to the thyroid-stimulating hormone receptor (TSHR). Stimulatory anti-TSHR antibodies activate thyroid cells, resulting in typical Graves’ hyperthyroidism. Although current treatment options are effective in initially eliminating the hyperthyroidism, they are not able to interrupt the autoimmune processes in Graves’ disease. Long-term eradication of hyperthyroidism in Graves’ disease patients could be achieved by antigen-specific immunotherapy as the formation of anti-TSHR antibodies is T cell dependent.

We designed a peptide-based antigen-specific immunotherapy to specifically re-establish immune tolerance to TSHR through the development of antigen-processing-independent epitopes (apitopes®). Combining MHC binding assays with immunization and tolerance induction experiments in human leukocyte antigen HLA-DRB1*0301 transgenic (DRtg) mice, TSHR immune dominant peptides were identified. The combination of these TSHR-derived peptides induced T cell tolerance towards TSHR in DRtg mice. In addition, a challenge model was created in DRtg mice using an adenovirus expressing the extracellular domain of TSHR. In this animal model, a mixture of two immunodominant apitopes® was sufficient to suppress the anti-TSHR antibody production by more than 90%. Thus, selected peptides efficiently regulate the anti-TSHR T and B cell responses, specifically the generation of anti-TSHR antibodies, in DRtg mice. Furthermore, selected peptides were assessed for their antigenicity using PBMC samples from Graves’ disease patients, demonstrating the identification of relevant human T cell epitopes. These results demonstrate that antigen-specific immunotherapy with apitopes® from TSHR is a suitable approach, and is currently undergoing clinical trials, for the treatment of Graves’ disease.

WS.C4.01.03  Pathogenic antibody development and blister formation following impaired peripheral tolerance

S. Haeberle1, X. Wei2, K. Bieber2, S. Galetz2, E. Schmidt2, R. Ludwig2, A. Enk2, E. Hadasschik1,2.

1Department of Dermatology, Heidelberg, Germany, 2Department of Dermatology, Lübeck, Germany.

Mutational functional regulatory T-cells (Treg) lead to development of severe autoimmune inflammation. Scurfy mice have a mutation in foxp3 and thereby no functional Treg. Scurfes show severe skin inflammation with Th1/Th2 prone immune response and high titers of autoantibodies against skin proteins. We wanted to analyze if autoimmune blistering diseases (AIBD) develop in the absence of Treg. We screened scurfy sera and found elevated levels of autoantibodies against different AIBD-related autoantigens. Histological analysis of a strong scurfy skin showed the presence of subepidermal blister and lymphocytes. Together with deposition of subepidermalsautoantibodies in the skin, we demonstrate that scurfies develop the phenotype of AIBD. We generated hybrids, to isolate monoclonal autoantibodies (mAb) from scurfes. Mass spectrometry analysis revealed BP230 as the autoantigen of one of these mAb (20B12). We found this antigen by immunofluorescence, western blot and ELISA. Furthermore, we proved pathogenicity by injection of 20B12 into neonatal mice. We transferred subepidermal blistering CD4+ T-cells from Scurfes and B-cells, CD4+ T-cells from scurfy and WT mice were transferred into nu/nu mice. Transfer of autoreactive scurfy CD4+ T-cells into nu/nu mice, resulted in autoantibody production and subepidermal blister formation similar to scurfy. In summary, we show that impaired peripheral tolerance leads to an AIBD phenotype with pathogenic autoantibody production and blister formation in scurfy mice. Furthermore the injection of anti-BP230 autoantibody was sufficient to induce blister formation, therefore BP230 should be considered as a relevant target autoantigen in AIBD. Funded by SFB/TR156.

WS.C4.01.04  APRIL induces a novel subset of Iga+ regulatory B cells that suppress inflammation through the expression of IL-10 and PD-L1

C. M. Fehres1, N. O. Van Uden1,2, N. G. Verenenko1,2, L. Fernandez1, G. Franco Salinas1, L. Van Duivenvoorde1,2, B. Huard1, J. Morel1, H. Skipitz1, M. Hahne1, D. L. Botten1,2,1,2

1Department of Clinical Immunology and Rheumatology, Academic Medical Center, Amsterdam, Netherlands, 2Department of Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands, 3Institute de Genetique Moleculaire de Montpellier, Centre National de la Recherche Scientifique, Universite de Montpellier, Montpellier, France.

Regulatory B cells (Bregs) are immunosuppressive cells that modulate complex immune responses through multiple mechanisms, such as the production of IL-10 and the skewing of T cell differentiation in favor of a regulatory phenotype. However, the signals required for the differentiation and activation of these cells remain still poorly understood. As we have previously found that APRIL induced IL-10 production in human B cells, we hypothesized that APRIL, but not BAFF, may be involved in the induction and/or activation of IL-10 producing Bregs that suppress inflammatory responses in vitro and in vivo. CD19+IgD-CD27 naive B cells were cultured in the presence of IL-21+TGF-β, IL-21+APRIL or IL-21+BAFF to induce class switch recombination to Iga+. Regulatory B cell functions and phenotypes were examined on the class switched Iga+ B cells. Here, we describe that APRIL promotes the differentiation of naive human B cells to IL-10-producing Iga+ B cells. These APRIL-induced Iga+ B cells display a Breg phenotype and inhibit T cell and macrophage responses in vitro through expression of IL-10 and PD-L1. Moreover, APRIL-induced IL-10 producing Bregs suppress inflammation in vivo in experimental autoimmune encephalomyelitis (EAE) and contact hypersensitivity (CHS) models. Finally, we showed a strong correlation between APRIL and IL-10 in the inflamed synovial tissue of inflammatory arthritis patients. In conclusion, we have identified a novel subset of regulatory B cells within the Iga+ B cell population that suppresses inflammation in vitro and in vivo, which indicate the potential relevance of this subset of B cells for immune homeostasis and immunopathology.

WS.C4.01.05  Molecular Mechanisms of T cell Tolerance Induction

S. T. N. Ng1, S. Bevington2, P. Cockerill3, D. C. Wraith4.

1University of Birmingham, Birmingham, United Kingdom.

Escalating peptide immunotherapy is effective in treating experimental autoimmune encephalomyelitis (EAE) in mice and multiple sclerosis in humans. EAE can be prevented and treated by subcutaneous administration of the peptide MBP Ac1-9[4Y] in H-2Kb transgenic (nu/nu) mice, resulted in autoantibody production and subepidermal blister formation similar to scurfy. In summary, we show that impaired peripheral tolerance leads to an AIBD phenotype with pathogenic autoantibody production and blister formation in scurfy mice. Furthermore the injection of anti-BP230 autoantibody was sufficient to induce blister formation, therefore BP230 should be considered as a relevant target autoantigen in AIBD. Funded by SFB/TR156.

WS.C4.01.06  Macrophages treated with exosome-delivered miRNA-150 release immune suppressive exosome-like nanovesicles bearing antigen/MHC complex

K. Nazimek1, B. Nowack1, M. Wasi1, W. Pakt1, P. W. Askene2, K. Brynias3,2.

1Department of Immunology, Jagiellonian University Medical College, Krakow, Poland, 2Section of Allergy and Clinical Immunology, Yale University School of Medicine, New Haven, United States.

Introduction. T cell-derived, miRNA-150-carrying exosomes suppress mouse contact (CHS) and delayed-type (DTH) hypersensitivity reactions (J Allergy Clin Immunol 2013;132:170-81). miRNA-150-carrying exosomes were shown to target antigen-presenting macrophages finally suppressing CHS effector cells [Immunology 2015;146:23-32]. Current studies are targeting the mechanism of immune suppression mediated by macrophages treated with exosome-miRNA-150. Methodology. Wild type, OT-I or miRNA-150 KO mouse peritoneal macrophages were treated with T cell-exosomes and standardly cultured for 48 hours in serum-free medium. Resulting supernatant was filtered down to 0.22micrometers and ultracentrifuged at 100.000g for 70 minutes. Pelleted nanovesicles were used for treatment of adoptively transferred DTH effector T cells or analyzed cytometrically. OT-II macrophage-derived exosome-like nanovesicles were pre-incubated with anti-ovabumin-323 antibodies. Ovabumin-derived DTH effector T cells of OT-I mice were re-challenged with Ovabumin. Results. MHC class II-positive exosome-nanovesicles from culture of miRNA-150-pulsed macrophages of wild type, OT-I or miRNA-150 KO mice suppressed adoptively transferred effector T cells, and this inhibitory effect was enhanced by pre-incubation of pelleted nanovesicles with ovabumin-323-specific antibodies and blocked by pre-incubation of effector T cells with ovabumin-323 peptide.

Conclusions. Macrophages release suppressive exosome-nanovesicles only after treatment with T cell-exosome-carried miRNA-150. As miRNA-150-pulsed macrophage-derived exosome-like nanovesicles express MHC class II and bind antigen-specific antibodies, we concluded that they display antigen/MHC complex. These exosome-like nanovesicles finally target effector T cells to suppress DTH immune response and this effect depends on the interaction of antigen/MHC complex expressed on macrophage-derived exosome-like nanovesicles with antigen-specific TCR of effector T cells.
WS.C4.02.01 IL-21 sustains atrophic and colitogenic CD4+ T cell effector responses by promoting apoptosis of Foxp3+ regulatory T cells

A. Freuchet, S. Bezie, C. Usal, V. Daguin, C. Guillonneau, I. Anegon; CRTI INSERM UMR 1064, Nantes, France.

Interleukin-21 (IL-21) has been shown to promote the expansion of CD4+ T cells, indicative of its contribution to the development of T helper (Th) cell responses. IL-21 also affects regulatory T (Treg) cells, which play a crucial role in the maintenance of immunological tolerance. The balance between the expansion and apoptosis of Tregs is tightly controlled, and the relative contributions of IL-21 to these processes remain under study. Our study aimed to investigate the effects of IL-21 on the expansion and apoptosis of Foxp3+ Treg cells, using mouse models of intestinal inflammation.

Mice lacking IL-21 were generated and used in the acute colitis model induced by dextran sodium sulphate (DSS), a model of Th17-driven inflammatory bowel disease (IBD). Mice were treated with IL-21 or vehicle control before DSS administration. The levels of Foxp3+ Treg cells were determined by flow cytometry in the colonic lamina propia and spleen.

Our results demonstrated a significant decrease in the number of Foxp3+ Treg cells in the colonic lamina propia of IL-21 knockout (KO) mice compared to wild-type (WT) mice. Furthermore, IL-21 KO mice showed increased expression of pro-inflammatory cytokines, such as interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α), indicating an exacerbated inflammatory phenotype. These findings suggest that IL-21 plays a critical role in maintaining Treg cell numbers and function in the gut, potentially by inhibiting apoptosis.

In conclusion, our study highlights the importance of IL-21 in the regulation of Treg cell homeostasis and suggests that therapies targeting IL-21 may be beneficial in the treatment of IBD, by promoting Treg cell survival and function.
WORKSHOPS

WS.C5.01 Physiopathology of allergic disorders

WS.C5.01.01 Facilitating C3aA-expressing platelets determine the severity of experimental asthma

F. Jansson1*, H. Beuter2, B. Hechler3, D. Godon4, Y. Wang1, C. M. Gillis5, L. de Chaisemartin6, A. Gouët-Chéron4, S. Magnenet7, L. Macdonald1, A. Murphy2, S. Chollet-Martin1

1Institut Pasteur/Inserm, Paris, France, 2IJSF Grand Esta/Inserm, Strasbourg, France, 3Hôpital Bichat (AP-HP)/Inserm, Paris, France, 4Regeneron Pharmaceuticals, New York, United States.

Platelets are key regulators of vascular integrity; however, their role in anaphylaxis, a life-threatening systemic allergic reaction characterized by the loss of vascular integrity and vascular leakage, is not well known. Anaphylatoxin C5a plays an important role in the development of maladaptive Th2/Th17 immunity in experimental allergic asthma through the activation of C5a Receptor-1 (C5ar1). Here, we characterized a pulmonary highly vacuolated SiglecF+ platelet subset that modulate IgE-mediated basophil activation. SiglecF+ platelets could be considered a biomarker and therapeutic target in IgE-mediated allergic diseases as it seems to be involved in the modulation of IgE-mediated basophil activation.

Funding: ERC, ANR-JCJC, Institut Pasteur, INSERM, Société Française d’Allergologie, Établissement Français du sang.

WS.C5.01.02 Novel role for the acute phase protein serum amyloid A in the initiation of type 2 immunity

U. Smole1*, S. Lajoie2, J. Vitallé3, I. Astigarraga1,2, P. König1,2, A. Hamann3,4, J. Walter1,2,4

1Institut Pasteur/Inserm, Paris, France, 2IJSF Grand Esta/Inserm, Strasbourg, France, 3Hôpital Bichat (AP-HP)/Inserm, Paris, France, 4Regeneron Pharmaceuticals, New York, United States.

Inhibitory C-type lectin receptors play a key role in regulation of immune cell activation and functions. Our group has previously demonstrated that SiglecF+ platelets can trigger by antibodies to generally ineffective antigens, resulting in a massive mediator release and rapidly occurring organ dysfunction. Human platelets express receptors for IgG antibodies and can release potent mediators, yet their contribution to anaphylaxis has not been previously addressed in mouse models. It is known that platelets do not respond to IgE antibodies. We have previously shown that platelet counts dropped immediately and dramatically at anaphylaxis induction, only when they expressed the human IgG receptor FcγRIIA. Platelet depletion attenuated anaphylaxis whereas thrombocytemia drastically worsened its severity. FcγRIIA-expressing platelets were directly activated by IgG immune complexes in vivo and were sufficient to restore susceptibility to anaphylaxis in resistant mice. Serotonin released by activated platelets contributed to anaphylaxis severity. Data from a cohort of patients suffering from drug-induced anaphylaxis indicated that platelet activation was associated with anaphylaxis severity and that anaphylaxis occurrence was accompanied by a reduction in circulating platelet numbers.

Our findings identify platelets as critical players of IgG anaphylaxis and provide a rationale for the design of platelet-targeting strategies to attenuate the severity of anaphylactic reactions.

Funding: EEC, ANR-JCJC, Institut Pasteur, INSERM, Société Française d’Allergologie, Établissement Français du sang.

WS.C5.01.03 CD300c receptor co-stimulates IgE-mediated basophil activation and its expression is increased in cow’s milk allergic children

O. Zenarruzabeitia Belaustegi1*, I. Vitalle1, I. Terrer1, A. Orrantia1, J. Astigarraga2,3, L. Dopazo4, C. González1, C. Tutusia1, L. Santos-Diez5, P. Gamboa1, A. Bilbao1,6, F. Borrega1,6

1BioCrues Health Research Institute, Barakaldo, Spain, 2Crues University Hospital, Barakaldo, Spain, 3University of the Basque Country, Leioa, Spain, 4Basurto University Hospital, Bilbao, Spain, 5Ikerbasque, Basque Foundation for Science, Bilbao, Spain, 6Basque Center for Transfusion and Human Tissues, Galdakao, Spain.

Basophils express high-affinity IgE receptors (FceRI) and their endogenous ligand, the acute phase protein serum amyloid A1 (SAA1), as major drivers of house dust mite (HDM)-induced IL-33 release in vitro and in vivo. Mechanistically, this involved the dissociation of biologically inert SAA1 oligomers into monomers that induce epithelial IL-33 release. In mice, local inhibition of FRP2 in the lungs or Saa1/2 deficiency abrogated HDM-induced airway hyperresponsiveness, IgE synthesis, bronchoalveolar lavage (BAL) eosinophilia, concomitant with reductions in Th2 cytokines, IL-13~IL-17c, and BAL IL-33 levels in allergic-exposed mice. This was dependent on SAA1 recognition by the cytosolic fatty acid binding protein (FABP) Der p 13 contained in HDM extract. Importantly our findings in mice translate to human allergic diseases. The FABP sensing pathway is upregulated in respiratory epithelial cells from chronic rhinosinusitis (CRS) patients resulting in increased IL-33 secretion associated with enhanced SAA monomer formation and FRP2 signaling. Taken together, we here report a novel mechanism of allergenicity which involves SAA1 facilitated allergen recognition via FRP2 leading to aberrant IL-33 release and type 2 responses. This novel paradigm allows for a new view on SAA1 as a potent driver of type 2 allergic immune responses at mucosal surfaces. Supported by the Austrian Science Fund FWF, NII and ATS.
WORKSHOPS

WS.C5.01.05 Single cell RNA-Seq identifies a unique pathogenic Th2 cell gene signature and pinpoints cellular metabolism as a major distinguishing factor
J. M. Coquet
Karolinska Institutet, Solna, Sweden.

T helper cells are critical to the development of asthma. In particular, Th2 cells are the main protagonists in asthma, although other T helper cell subsets are known to participate in the regulation of asthma severity. Although decades of research have elucidated a number of factors, T helper cell differentiation, gene expression profiles of T helper cells typically come from arrays of bulk cell populations from in vitro-differentiated cells. We performed single cell RNA-Sequencing on T helper cells from the airways of mice administered house dust mite antigens, in an attempt to obtain pure transcriptional profiles of T helper cell subsets, in particular the pathogenic Th2 cell population. Our results indeed clearly depict populations of Th2, Treg and Th1 cells. In addition, we characterize a population of type I IFN responding cells and a population of activated T helper cells, expressing mRNAs for ribosomal proteins. Th2 cells indeed expressed Gata3, Il4, Il13 and Il25 as expected, but were also highly enriched for the expression of approximately 100 other genes, many of whose functions are not typically associated with Th2 cells. Pathway analysis identified metabolic processes as the major point of difference between Th2 cells and other T helper cells in the airways, in particular, those relating to fatty acid oxidation and synthesis. Taken together, our data shows that Th2 cells differ substantially from other T helper cells in a mouse model of asthma, with cellular metabolism being a major point of difference. Work characterizing human Th2 cells is ongoing.

WS.C5.01.06 Notch signaling in CD4+ T cells supports lymph node egress of Th2 cells in allergic airway inflammation
I. Tindemans1, A. KleinJan, M. J. de Bruin2, M. Lukkes2, V. F. van Ijcken2, D. Amers2, R. Staalhouders1, R. W. Hendriks2

Background: Notch signaling controls T cell differentiation through direct induction of Gata3. However, the role of Notch signaling during Th2-mediated atopic asthma is currently unclear.

Methods: We used a house-dust mite (HDM)-driven model to investigate the capacity of Notch1 and Notch2-deficient T cells to induce AAI. The role of Notch signaling in vivo and in vitro was studied using Notch-deficient ovabumin-specific T cells. We performed transcriptome analysis of T cells to identify genes that are controlled by Notch signaling in the context of AAI.

Results: In the HDM-treated groups, WT animals developed AAI, but conditional Notch1/Notch2 double KO animals failed to develop eosinophilic inflammation, and Th2 cytokines or serum IgE were not induced. When conditional Notch KO mice which concomitantly expressed a CD2-Gata3 transgene were treated with HDM, only a partial enhancement of Th2 inflammation was observed. Surprisingly, when Notch signaling is not required for the induction of Th2 differentiation. However, during the allergen challenge phase Notch-deficient T cells did not cause AAI, as they accumulated in draining lymph nodes and did not efficiently migrate to the lung. Transcriptome comparisons of Notch-deficient and WT Th2 cells from lymph nodes revealed 692 differentially expressed genes, including genes involved in cell migration and adhesion.

Conclusion: Notch signaling in T cells is crucial during Th2-mediated AAI in a HDM-driven asthma model, likely by mediating lymph node egress of Th2 cells.

WS.C5.02.01 Selective elimination of allergen-specific B lymphocytes with chimeric protein-engineered molecules
N. R. Ralchev1, N. Mihaylova1, N. Kerekov2, A. Tchorbanov1

1Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, and 2National Institute of Immunology, Sofia, Bulgaria.

Introduction: Der p1 is an allergenic molecule of Dermatophagoides pteronyssinus (Dpt) which causes house dust mite (HDM) allergy. The pathological Der p1-specific B cells produce allergen-specific IgE antibodies that mediate most of the hypersensitivity allergy reactions. It may be possible to influence Der p1-specific B cells in mouse model of HDM allergy by administration of chimeric molecule, containing a monoclonal antibody against the inhibitory B-cell receptor FcγRIIB coupled to a B and a T cell epitope-carrying peptides from the Der p1 peptide. Co-crosslinking of the immunoglobulin receptors and FcγRIIB by this molecule is expected to deliver strong suppressive signal selectively silencing these B cells and the subsequent allergic response. Materials and Methods: protein engineering, FACS, animal model, immunohistochemistry, histology. Results: A protein engineered chimeric molecule has been constructed, which binds Der p1 specific B-cells via their BCR and suppresses selectively the production of anti-Der p1 antibodies via FcγRIIb. The synthetic protein-engineered chimeric molecule has been tested for the construction of Der p1 chimeras. The functional effects of engineered antibodies were analyzed in vitro. An animal model of HDM allergy has been developed. Significant inhibition of allergen-specific proliferation and reduction of Derp1-IgE antibody production were observed after treatment of spleenocytes from sick mice with Derp1-peptide chimera. Conclusions: The present study explores a different approach for preventing pathological allergen-specific IgE antibody production. Our data show that the allergic immune response can be selectively silenced by targeting the pathological B cell.

WS.C5.02.02 Sulfitized Non-Anticoagulant Heparin (S-NACH) blocks allergen induced asthma manifestations in mice by modulating the IL-4/STAT6/GATA-3 pathway harboring a therapeutic potential for allergic asthma
M. A. Ghonim1, J. Wang2, S. Ibbat3, K. Pykure4, H. Liu5, I. Beslimane4, S. Mousa6, H. Boulware7

1Louisiana State University Health Sciences Center, New Orleans, LA, United States, 2Albany College of Pharmacy and Health Sciences, the Pharmaceutical Research Institute, Albany, NY, United States.

Background: The efficacy of heparins and Low-MW-heparins (LMWH) against human asthma has been known for decades. However, the clinical utility of these compounds has been hampered by their anti-coagulant properties. Much effort has been made to harness the anti-inflammatory of LMWH but so far none have gone to be used as therapy for asthma. Sulfated Non-Anticoagulant Heparin (S-NACH), an ultrasulufated heparin with no systemic anticoagulant effects. The present study explored the potential of S-NACH in blocking allergic asthma. Methods: Acute and chronic ovabumin-based mouse models of asthma, splenocytes, and a lung epithelial cell line were used. Mice were challenged to aerosolized ovabumin. Mice were administered S-NACH or saline 30 min after each ovabumin challenge. Results: S-NACH administration in mice promoted a robust reduction in airway eosinophilia, mucous production and airway hyperreactiveness even after chronic repeated challenges with ovabumin. Such effects were linked to a suppression of the Th2 cytokines IL-4/Il-5/IIL-13/GM-CSF and ovabumin-specific IgE without any effect on IFN-γ. S-NACH also reduced lung fibrosis in chronically ovabumin-exposed mice. These protective effects of S-NACH may be attributed to modulation of the IL-4/STAT6 signal transduction pathway through an inhibition of STAT6 and a subsequent inhibition of GATA-3 and Inducible NO synthase expression. S-NACH treatment also reduced the basal expression of the two isoforms of Arginase Arg1 and Arg2 in lung epithelial cells. Conclusions: Our results demonstrate that vEOS serve as additional antigen-presenting cells in experimental allergic asthma.

Collectively, our data show that vEOS exert strong antigen-presenting properties and induce AAI. They activate T cells and other T helper cells in the airways, in particular, those relating to fatty acid oxidation and synthesis. Taken together, our data shows that Th2 cells differ substantially from other T helper cells in a mouse model of asthma, with cellular metabolism being a major point of difference. Work characterizing human Th2 cells is ongoing.

Conclusion: In vivo, the role of Notch signaling controls T cell differentiation through direct induction of Gata3. However, the role of Notch signaling during Th2-mediated atopic asthma is currently unclear. We used a house-dust mite (HDM)-driven model to investigate the capacity of Notch1 and Notch2-deficient T cells to induce AAI. The role of Notch signaling in vivo and in vitro was studied using Notch-deficient ovabumin-specific T cells. We performed transcriptome analysis of T cells to identify genes that are controlled by Notch signaling in the context of AAI.

Results: In the HDM-treated groups, WT animals developed AAI, but conditional Notch1/Notch2 double KO animals failed to develop eosinophilic inflammation, and Th2 cytokines or serum IgE were not induced. When conditional Notch KO mice which concomitantly expressed a CD2-Gata3 transgene were treated with HDM, only a partial enhancement of Th2 inflammation was observed. Surprisingly, when Notch signaling is not required for the induction of Th2 differentiation. However, during the allergen challenge phase Notch-deficient T cells did not cause AAI, as they accumulated in draining lymph nodes and did not efficiently migrate to the lung. Transcriptome comparisons of Notch-deficient and WT Th2 cells from lymph nodes revealed 692 differentially expressed genes, including genes involved in cell migration and adhesion.

Conclusion: Notch signaling in T cells is crucial during Th2-mediated AAI in a HDM-driven asthma model, likely by mediating lymph node egress of Th2 cells.

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Neutrophils negatively regulate T2 inflammation in allergic airways disease by limiting G-CSF-driven monocyte and IL22 cytokine production

Imperial College London, London, United Kingdom

Neutrophils are critical components of the body’s anti-microbial response, yet an over-exuberant neutrophilic inflammation has traditionally been implicated in the pathology of chronic diseases. Increasingly, however, it is recognized that neutrophils are not just indiscriminate killers but are able to elegantly and specifically shape many facets of the elicited immune response. Vastly circumscribed evidence has suggested a prominent role for neutrophils in the pathophysiology of severe asthma, but their precise contribution to disease progression is poorly understood. In a house dust mite model (HDM) of allergic airways disease (AAD), we demonstrate that neutrophil depletion unexpectedly resulted in exacerbated T2 inflammation, with increased numbers of T2 CD4+ T-cells and type 2 innate lymphoid cells (ILC2s) and elevated levels of allergen-specific IgE and IgG1 antibodies. This augmented T2 inflammation in neutrophil-depleted mice was preceded by an early increase in levels of IL22-derived IL-5 and IL-13 and numbers of monocyte-dervied dendritic cells (MoDCs) and ensuing antigen presentation. Central to the exacerbated T2 inflammation in neutrophil-depleted mice was a striking increase in G-CSF levels, which drove an expansion on monocyte progenitors in the bone marrow and T2 cytokine production by ILC2s in the airways. In conclusion, we demonstrate that neutrophil depletion during AAD results in profound perturbations in the G-CSF axis and an ensuing monocyte and IL22 driven exacerbation in T2 inflammation. These studies reveal a novel role for neutrophils in negatively regulating monocyte and IL22 function and highlight potential complexities of targeting neutrophils in animal models and within the clinic.

Neutrophils negatively regulate T2 inflammation in allergic airways disease by limiting G-CSF-driven monocyte and IL22 cytokine production

Imperial College London, London, United Kingdom

Neutrophils are critical components of the body’s anti-microbial response, yet an over-exuberant neutrophilic inflammation has traditionally been implicated in the pathology of chronic diseases. Increasingly, however, it is recognized that neutrophils are not just indiscriminate killers but are able to elegantly and specifically shape many facets of the elicited immune response. Vastly circumscribed evidence has suggested a prominent role for neutrophils in the pathophysiology of severe asthma, but their precise contribution to disease progression is poorly understood. In a house dust mite model (HDM) of allergic airways disease (AAD), we demonstrate that neutrophil depletion unexpectedly resulted in exacerbated T2 inflammation, with increased numbers of T2 CD4+ T-cells and type 2 innate lymphoid cells (ILC2s) and elevated levels of allergen-specific IgE and IgG1 antibodies. This augmented T2 inflammation in neutrophil-depleted mice was preceded by an early increase in levels of IL22-derived IL-5 and IL-13 and numbers of monocyte-dervied dendritic cells (MoDCs) and ensuing antigen presentation. Central to the exacerbated T2 inflammation in neutrophil-depleted mice was a striking increase in G-CSF levels, which drove an expansion on monocyte progenitors in the bone marrow and T2 cytokine production by ILC2s in the airways. In conclusion, we demonstrate that neutrophil depletion during AAD results in profound perturbations in the G-CSF axis and an ensuing monocyte and IL22 driven exacerbation in T2 inflammation. These studies reveal a novel role for neutrophils in negatively regulating monocyte and IL22 function and highlight potential complexities of targeting neutrophils in animal models and within the clinic.

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Moreover, we identified a molecular link between GC and PPAR-γ and could show for the first time that PPAR-γ modulates GC-induced migration in macrophages. In conclusion, we could demonstrate that PPAR-γ exerts anti-inflammatory activities and shapes macrophage functions.

inflammatory phenotype upon long-term LPS stimulation and showed impaired phagocytosis compared to WT cells. GC treatment of macrophages led to the upregulation of PPAR-γ and could also demonstrate that CD38 expression in macrophages depends on PPAR-γ. As expected, PPAR-γ KO macrophages displayed an increased pro-inflammatory response to lipopolysaccharide and glucocorticoids.

Increased recruitment to the site of inflammation. In conclusion, we could demonstrate that PPAR-γ exerts anti-inflammatory activities and shapes macrophage functions.

PPAR-γ modulates macrophage response to lipopolysaccharide and glucocorticoids.

VISTA expression in mouse microglia was accompanied by decreased acetylation of lysine residue 27 in histone 3 in both its promoter and enhancer region. ATAC-sequencing expression by Pu.1 and Mafb, two transcription factors crucial for microglia function. Finally, our data suggested that VISTA expression was reduced in mouse microglia during inflammation and is differently regulated in CNS diseases.

Expression changes of other NRs (PD-1, PD-L1/2, CTLA-4) during inflammation of the central nervous system (CNS) were previously demonstrated, but VISTA expression in the CNS has not yet been explored. Here, we report that in the human and mouse CNS, VISTA is most abundantly expressed by microglia, and to lower levels by endothelial cells. Upon TLR stimulation, VISTA expression was reduced in primary neuronal mouse and adult human microglia in vitro. In mice, microglial VISTA expression was reduced after lipopolysaccharide (LPS) injection, during experimental autoimmune encephalomyelitis (EAE), and in the accelerated aging Clec12a KO mouse model. After LPS injection, decreased expression in mouse microglia was accompanied by decreased acetylation of lysine residue 27 in histone 3 in both its promoter and enhancer region. ATAC-sequencing indicated a potential regulation of VISTA expression by Pu.1 and Mafb, two transcription factors crucial for microglia function. Finally, our data suggested that VISTA expression was reduced in microglia in multiple sclerosis lesion tissue, whereas it was increased in Alzheimer’s disease patients. We are currently analysing VISTA expression in different types of MS lesions in detail. This study is the first to demonstrate that in the CNS, VISTA is expressed by microglia, and that VISTA is differentially expressed in CNS pathologies.

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Inhibition of NETosis (WT) and KO neutrophils release increased ROS and NETs in response to stimulation. Inhibition of NETosis in vivo, via treatment with BB-CL-Amidine, reduced joint inflammation in Clec12a KO mice. While we did not find any association of polymorphisms in Clec12a with disease, a subset of RA patients presented with autoantibodies against CLEC12A in their sera. Importantly, administration of anti-CLEC12A antibodies to WT mice induces the same exacerbation of inflammation seen in the KO mice. Together these data suggest a novel mechanism whereby autoantibodies may interfere with an inhibitory pathway regulating neutrophil responses in the initiation of RA.

Clec12a regulates neutrophil functioning in Rheumatoid arthritis

Clec12a regulates neutrophil functioning in Rheumatoid arthritis.

A role for DPP4 in T cell-mediated vascular inflammation and Atherosclerosis

VISTA expression in microglia decreases during inflammation and is differently regulated in CNS diseases.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Identification of regulatory mechanisms that influence inflammation in metabolic tissues, including adipose and liver, are critical to developing treatments for metabolic diseases such as obesity and diabetes. Here, we investigated the protective role of microRNA-146a (miR-146a) during diet-induced obesity (DIO). This microRNA has been shown to be reduced in obese and type 2 diabetic patients and to regulate inflammation in other contexts. Results revealed that miR-146a-/− mice fed a high-fat diet (HFD) have increased weight gain and adiposity. Reduced miR-146a correlated with increased expression and activation of the pro-inflammatory cytokine IL-6, and diet-induced hyperglycemia and hyperinsulinemia. We demonstrate for the first time that miR-146a regulates inflammation through its modulation of key genes that control inflammation and insulin sensitivity. Altogether, these data demonstrate important functions for miR-146a in preventing obesity and metabolic disease by regulating immunometabolic pathways within macropores.

Training for this project was funded by an NIH T32 in Metabolism and Diabetes, awarded to M.R.

WS.C6.02.03 IFNβ is a novel effector cytokine in resolving inflammation

A. Arief1, S. K. Satyanarayanan2, S. Sobah1, S. Butenki1, J. Saoda1, A. Kossi1, N. Pelled1, N. Sher1, S. Schif-Zuck1

1University of Haifa, Haifa, Israel, 2The Tauber Bioinformatics Research Center, University of Haifa, Haifa, Israel.

The engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for tissue homeostasis and results in macrophage reprogramming/lineage-silencing. However, a distal control of resolution phase macrophages loss their phagocytic potential, and hence were termed as reduced patrolling macrophages. Here, we show using an unbiased RNA-Seq analysis that saturated macrophages express a distinct IFNβ-related signature. Unexpectedly, we found peritoneal IFNβ levels peaked during the onset as well as the resolution phase of peritonitis. Consequently, we determined IFNβ limited the onset of neutrophilic inflammation by enhancing PMN apoptosis. Moreover, IFNβ enhanced macrophage efferocytosis and reprogrammed to a pro-resolving phenotype. These findings indicate for the first time that IFNβ is a key effector cytokine in resolving inflammation.

WS.C6.02.04 CXCL9(74-103) reduces neutrophil recruitment and joint inflammation in experimental arthritis

D. Doff1, H. Crijs2, R. Janssens2, V. Vanheule2, G. B. Meneses3, S. Macor1, T. A. Silke1, F. A. Armar2, P. Proost4

1Instituto de Ciencias Biológicas, UFMG, Belo Horizonte, Brazil, 2Rega Institute, KU Leuven, Leuven, Belgium, 3Instituto de Biología Gastrointestinal, UFMG, Belo Horizonte, Brazil, 4Faculdade de Odontologia, UFMG, Belo Horizonte, Brazil.

The effect of the C-terminal CXCL9-derived peptide, CXCL9(74-103), on joint inflammation was investigated in a murine model of antigen-induced arthritis (AIA) in male C57BL/6 mice. Simultaneous intravenous injection of CXCL9(74-103) with a biotin maleimido-modified chelating bovine serum albumin (mBiSA) as antigen in mice immunized with mBiSA, resulted in a reduced accumulation of leukocytes, mainly neutrophils, in the lungs compared to WT controls. Pro-inflammatory gene expression, TNFα, IL-1β, and IL-6 strongly increased, and IL-10-like type II interleukin (IL-6) were decreased in mice treated with CXCL9(74-103) compared to non-treated AIA mice. In addition, cartilage and bone damage were substantially reduced upon CXCL9(74-103) treatment. CXCL9(74-103) competes with CXCL6 and CXCL13 for binding to glycosaminoglycans in vitro. Confoal microscopy allowed to visualize rapid binding of CXCL9(74-103) to blood vessels in joints. Delayed (up to 6 hours) treatment with CXCL9(74-103) still reduced neutrophil accumulation in the joint, but did not affect chemokine and cytokine concentrations in the serum. Thus, CXCL9(74-103) peptide controlled the massive accumulation of neutrophils in the joints of AIA mice through inhibition of the interaction between chemokines and glycosaminoglycans and diminished tissue damage. This research was supported by KU Leuven, the Hercules Foundation, CPNP Brazil and FWO Vlaanderen. H. C. holds a SB PhD fellowship of the FWO Vlaanderen, Belgium.

WS.C6.02.05 Inhibition of neutrophil extravasation with a CXCL9-derived glycosaminoglycan-binding peptide

P. Proost1, V. Vanheule1, A. Mortier1, H. Crijs1, D. Doff1, R. Janssens2, S. Struyf1, F. A. Armar2, M. M. Teixeira2

1KU Leuven, Rega Institute, Leuven, Belgium, 2UFGM, Belo Horizonte, Brazil.

Chemokines are presented on the endothelium and immobilized in the tissue at the site of inflammation through binding to glycosaminoglycans (GAGs). This interaction ensures high local concentrations of the produced chemokines, prevents their diffusion and inhibits proteolytic degradation. Subsequently, chemokines interact with their G-protein coupled receptor(s) (GPCRs), which results in adhesion of leukocytes and extravasation through the endothelium. The binding of chemokines to GAGs has been proven indispensable for chemokine activity in vivo. We discovered that the COOH-terminal tail of CXCL9 has potent GAG binding properties. In addition, synthetic CXCL9-derived peptides competed with active chemokines for GAG binding in an ELISA-like heparin binding assay and inhibited the in vivo recruitment of neutrophils towards the major human neutrophil attractant IL-8 or CXCL8 injected in the peritoneum or the knee cavity of mice. Reducing the length of the CXCL9 peptide, especially when one of the GAG-binding motifs was deleted, gradually decreased the capacity to compete with CXCL9 for GAG binding. Using intravital microscopy, we showed that the CXCL9 peptide coasts the endothelial cell surface of blood vessels in vivo thereby inhibiting CXCL8-induced neutrophil adhesion in the murine cremaster muscle model. Intravenous application of the CXCL9-derived peptide resulted in inhibition of MSU crystal-induced neutrophil migration to the knee cavity in a mouse model of gout. In conclusion, we identified an alternative approach for inhibitors of chemokine - GPCR interactions to target the chemokine system to reduce inflammation.

WS.C6.02.06 Mechanisms by which lung surfactant protein-SA amplifies IL-4-mediated effects on alveolar macrophages

C. Casals1, J. B. García-Fojeda2, C. Montero-Fernández2, C. Stamm1, C. M. Minut1

1Complutense University of Madrid, Madrid, Spain, 2Biochemical Center of Respiratory Diseases (CIBERES), Madrid, Spain, 1Leibniz Center for Medicine and Biosciences, Borstel, Germany, 2University of Lübeck, Lübeck, Germany, 3The Francis Crick Institute, London, United Kingdom.

The phenotype of alveolar macrophages (AMs) is determined in part by the surfactant environment. We recently reported that AMs switch their phenotype by integrating IL-4 and lung-specific signals that lead to activation of tissue repair programs. Surfactant protein SA is a lung factor that amplifies IL-4A-dependent activation and proliferation of AMs via myosin18A receptor (Myo18A). However, the mechanism by which IL-4 and IL-4 synergistically enhance activation and proliferation of AMs remains elusive. Here we show that SP-A activated PI3K through binding to Myo18A receptor, accordingly, blocking PI3K activity or Myo18A receptor abrogated SP-A’s effects on IL-4 signaling. SP-A-dependent activation of PI3K and subsequent phosphorylation of its downstream effectors Akt, TSC-mTORC1, GSK3β, and PKCβ amplified IL-4-mediated AM proliferation and/or alternative activation. On the one hand, SP-A sustained the PI3K-Akt-mTORC1 signaling pathway triggered by IL-4. Both alternative activation and proliferation of AMs induced by SP-A + IL-4 are inhibited by Akt inhibitor VIII, torin1 (mTORC1/mTORC2 inhibitor), and rapamycin (mTORC1 specific inhibitor). Our results also indicate that SP-A increased Akt-dependent phosphorylation of GSK3β, which abrogates its role in inhibiting proliferation. On the other hand, the SP-A/Myo18A/PI3K/PKCβ axis was involved in enhancing IL-4-dependentSTAT6 activation and arginase activity. PKCβ inhibition by PKCβ pseudosubstrate was able to reduce IL-4+SP-A-driven pSTAT6 and alternative activation, but not proliferation. In conclusion, SP-A activates PI3K-dependent coordinated signaling pathways that allow IL-4 actions in cell proliferation and activation of effector functions. This study was supported by Spanish Ministry of Economy and Competitiveness (SAF2015-65307-R) and Institute of Health Carlos III (CIBER CB06/060002).

WS.C6.03.01 Plasmacytoid dendritic cells promote lung and skin fibrosis

R. S. Singh1, S. Kafaja1, A. Diversek2, R. Sagar3, A. Thibaz4, D. Khanna1, D. Furst5, I. Valera5

1David Geffen School of Medicine, UCLA, Los Angeles, United States.

Fibrosis is the end-result of most inflammatory conditions, but its pathogenesis remains unclear. Here, we investigated the role of plasmacytoid dendritic cells (pDCs) in the pathogenesis of cutaneous fibrosis using the murine bleomycin-induced lung and skin fibrosis model and clinical samples from patients with systemic sclerosis (SSc). We demonstrate that in animals and humans with systemic fibrosis, pDCs are unaffected or reduced systemically (spleen/peripheral blood) but they increase in the affected organs (lungs/skin/ bronchoalveolar lavage). Depletion of pDCs in animals ameliorated bleomycin-induced lung and skin fibrosis, and reduced infiltration with B-cells, T-cells, and natural killer T-cells in the affected organs but not in spleen. pDC-depleted bleomycin-injected mice also had a reduced expression of genes and proteins involved in chemotaxis, dendritic cell differentiation, inflammation, and fibrosis in the lungs as compared to controls. In resonance with animal findings, the frequency of pDCs in the lungs of patients with SSc correlated with the severity of lung disease, and with the frequency of CD4+ and IL-4+ T-cells in the lung. Finally, treatment with a tyrosine kinase inhibitor imatinib has been reported to reduce and/or prevent deterioration of skin and lung fibrosis profoundly reduced pDCs in lungs but not in peripheral blood of patients with systemic sclerosis. These observations suggest a role of pDCs in the pathogenesis of systemic fibrosis and identify the increased trafficking of pDCs to the affected organs as a potential therapeutic target in fibrotic diseases.
WS.C6.03.02
S100A8/A9 promotes parenchymal damage and renal fibrosis in obstructive nephropathy
A. Tomaras
AMC, Amsterdam, Netherlands.

Despite advances in our understanding of the mechanisms underlying progression of chronic kidney disease and the development of fibrosis, only limited efficacious therapies exist. The calcium binding protein S100A8/A9, is a damage-associated molecular pattern which can activate TLR4 or RAGE. Activation of these receptors is involved in the progression of renal fibrosis, however, the role of S100A8/A9 herein remains unknown. Therefore, we analysed S100A8/A9 expression in patients and mice with obstructive nephropathy and subjected wild-type and S100A8 KO mice, lacking the heterodimer S100A8/A9, to Unilateral Ureteral Obstruction (UUO). We found profound S100A8/A9 expression in granulocytes that infiltrated human and murine kidney, and enhanced renal expression over time following UUO. S100A8 KO mice were protected from UUO-induced renal fibrosis, independently of leukocyte infiltration and inflammation. Loss of S100A8/A9 protected tubular epithelial cells from UUO-induced apoptosis and critical epithelial-mesenchymal transition steps. In vitro studies revealed S100A8/A9 as a novel mediator of epithelial cell injury, through loss of cell polarity, cell-cycle arrest and subsequent cell death. In conclusion, we demonstrate that S100A8/A9 mediates renal damage and fibrosis presumably through loss of tubular epithelial cell contacts and irreversible damage. Suppression of S100A8/A9 could be a therapeutic strategy to halt renal fibrosis in patients with chronic kidney disease.

WS.C6.03.03
CXCL4 drives inflammation and fibrosis through monocytic-derived dendritic cells through transcriptional and epigenetic imprinting
1-University Medical Center, Utrecht, Utrecht, Netherlands; 2-Theoretical Biology group, University Utrecht, Utrecht, Netherlands.

Accumulation of extracellular matrix (ECM) or fibrosis is one of the hallmarks that characterizes the pathogenesis of systemic sclerosis (SSc), together with immune dysregulation and small vessel vasculopathy. CXCL4 (Chemokine CXCl motif ligand 4) levels are increased in SSc patients and correlated with skin and lung fibrosis. We and others shown that CXCL4 modulates phenotype and function of immune cells, however how CXCL4 modulates immune cell responses remains unclear. Here we investigated the impact of CXCL4 exposure on the transcriptome and DNA methylation during monocyte-derived dendritic cells (moDCs) differentiation and stimulation with polyC. Integration of high-throughput data reveals that CXCL4 drives to dramatic changes on the transcriptome and DNA methylation. This is reflected in the dysregulated of metabolic pathways, HIF-1 signaling, innate and adaptive immune pathways. Also, CXCL4 potentiates a novel function to moDCs, namely the production of ECM molecules, such as fibronectin (FN1) and TGFβ1. CXCL4 exposure results in epigenetic imprinting during moDC differentiation and gene regulatory network analysis reveal that CXCL4 regulates key transcriptional regulators. In conclusion, we show that CXCL4 besides driving to dramatic changes on moDC phenotype and function, this chemokine is the first endogenous ligand that leads to innate immune tumor infiltration, we find for the first time the direct regulation of CXCL4 on the production of ECM by moDCs, thereby characterizing the role of CXCL4 in inflammatory and fibrotic conditions such as SSc. Supported by: Portuguese FCT No.SFRHI/BD/89643/2012 (SCC); China Scholarship Council (CSC) No.201606030050 (WT); ERC starting grant (CIRCUMVENT) and Arthritis foundation grant (TRD/JIR).

WS.C6.03.04
Liver sinusoidal endothelial cells trigger CDB T cell mediated liver failure and herald liver regeneration
1-Institute of Molecular Immunology and Experimental Oncology, Technical University of Munich, Munich, Germany, 2-German Cancer Research Center, Heidelberg, Germany.

Introduction: Liver-sinusoidal-endothelial-cells (LSECs) have unique immune features that enable them to coordinate local hepatic immune responses. Recently, we showed that LSECs can directly promote hepatitis through and upregulation of pro-inflammatory (LSEC-Like) cytokines. This promotes the recruitment of pro-inflammatory lymphocytes and macrophages and triggers liver failure. Here, we address whether LSECs also contribute to liver regeneration by HGF-expression. Material and Methods: Primary cell cultures of LSEC were established to investigate HGF-expression, proteomics/secretomics of in vitro stimulated LSECs for determination of their pro-regenerative potential, in vitro LSEC cross-presentation to T cells and its contribution to HGF production. Results: Exposing LSECs to dying hepatocytes, extracellular ATP, or other P2X7 receptor ligands failed to induce HGF-production excluding a sentinel role for local cell death promoting liver regeneration. However, LSECs produced HGF after IL-6 cluster-signalling in a dose-dependent fashion. Since IL-6 cluster-signalling is induced in LSECs upon mutual activation during cross-presentation to CDB T cells, HGF levels were also increased after LSEC-activation of CDB T cells. LSEC Proteome/secretome analysis revealed that upon IL-6 cluster-signalling pro-HGF levels decreased and levels of matured HGF increased, consistent with increased enzymatic activity upon IL-6 cluster-signalling of e.g. prokinease, to convert inactive pro-HGF into bioactive matured HGF. Conclusion: We discovered a pivotal role for LSEC in triggering T cell-mediated liver damage but also promoting liver regeneration through production of bioactive HGF. Generation of bioactive HGF by LSECs in response to local T cell activation may act in a pre-emptive fashion to kickstart liver regeneration already at the time of impending immune-mediated liver damage.

WS.C6.03.05
NLRP3- A key in the mediation of colitis into colitis-associated colorectal cancer
A. P. Perera, R. Eri;
1-Shool of Health Sciences, Launceston, Australia.

Ulcerative colitis is a known risk for development of colorectal cancer but the exact mechanism of how chronic inflammation induce cancer has not been established. Many studies have indicated a role for NLRP3 inflammasome in colitis and tumorigenesis but the results have been controversial due to different chemical models of colitis induction and altered microbtiota. To address the inconclusive role of NLRP3 we have designed the first murine model deficient in NLRP3 in a spontaneous chronic colitis mouse model Winnie (Mac2 mutant). Extensive studies done in Winnie has proven it to be the most available murine model to study ulcerative colitis and its pathogenesis. Our results show colon tumorigenesis in Winnie mice with both high burden of colonic polyps starting at 12 weeks. NLRP3+/- Winnie mice have significantly shorter colon, and a higher ratio of colon weight to length indicating the severity of colitis. Histological examination NLRP3−/− Winnie colon revealed differentiated adenocarcinoma with high-grade dysplasia and hyperplasia regions. Extracted RNA from colonic segments was used for analysis of colorectal cancer biomarker gene expression using PCR micro array. Colon organ culture supernatants were assayed via Bio-Plex for proinflammatory cytokines. Flow cytometry was done to immunophenotype T cells, B cells, neutrophils and natural killer cells in WinniesNLRP3−/−. We analysed NLRP3−/− Winnie faecal metabolomics and characterized the faecal microbiota with 16S RNA to identify specific microbial signature of colitis associated cancer. Our results describe the role of NLRP3 inflammasome in colorectal cancer leading to the development of novel therapeutic tools.

WS.C6.03.06
The IgE-FcεRI axis strongly promotes epithelial hyperplasia and inflammation-driven skin carcinogenesis
M. Hayes, S. Ward, L. Wang, R. Castro Seoane, G. Crawford, J. Strid;
1-Imperial College, London, United Kingdom.

The skin is a potent site for induction of type 2 immunity and IgE. Previous work has shown that accumulation of IgE effector cells in the skin is a consistent hallmark of skin perturbation and inflammation. Little is however known about the physiological role of IgE-carrying cells in the skin. Here, we examine the role of IgE and IgE effector cells in skin inflammation and epithelial carcinogenesis driven by chronic inflammation. We show that the inflammation-driven outgrowth of skin tumours is highly dependent on IgE and FcεRI effector cells. Mice lacking IgE or FcεRI developed significantly fewer and smaller tumours in a two-stage chemical carcinogenesis model. During the ‘tumour-promoting’ skin inflammation, high numbers of IgE-carrying basophils and mast cells accumulated in the skin whereas basophils selectively also infiltrated the tumours. Skin basophils were more immune active than mast cells and expressed high levels of IL-4, IL-6, IL-13, histamine and prostaglandins. The production of type 2 cytokines, as well as release of histamine was critically dependent on IgE and FcεRI. Further in vivo and in vitro analysis demonstrated that basophils, in an IgE-dependent manner, induced keratinocyte activation, proliferation and differentiation. Similar results were obtained in a primary keratinocyte cell line cultured with histamine. Collectively, these data demonstrate that the keratinocyte response was mainly induced via engagement of histamine receptors. Collectively, this work demonstrate that IgE effector cells, predominantly basophils, exacerbate skin inflammation, induce epidermal hyperplasia and play a key role in promoting inflammation-driven carcinogenesis.
WS.D1.01.01 Muruscular immune regulation

**WS.D1.01.01**

**Transcription factor c-Maf is required for the control of Th17 cell responses by intestinal regulatory T cells**

H. Hussein, S. Denanglaire, Y. Ajououaou, O. Lea, F. Andris; Laboratory of Immunobiology, Gosselies, Belgium.

Intestinal Tregs selectively inhibit immune responses directed against commensal microorganisms and allow responses against pathogens, thereby maintaining intestinal homeostasis and avoiding chronic inflammatory subsets of Tregs whose phenotype and function are strongly influenced by their environment. In contrast to most splenic Tregs, intestinal Tregs express high levels of c-Maf, a bZIP transcription factor involved in the differentiation and function of multiple helper T cell subsets.

Ablation of c-Maf in Tregs results in the development of spontaneous colitis, characterized by a rectal prolapse, increased Th17-associated cytokine expression and expansion of Th17 cells in the intestine. The increased Threg percentage suggests that the spontaneous colitis is a consequence of an altered Threg function rather than a quantitative defect.

**ROrRt** Tregs are induced in the intestine by the microbiota. Their colocalization with Th17 cells and high IL-10 expression levels makes them particularly apt at controlling Th17 responses. Our results show the loss of ROrRt Tregs in the intestine of c-Maf deficient mice, suggesting a selective role for c-Maf in the differentiation of this subset. Current investigations aim to decipher the signaling pathways leading to c-Maf expression in gut-associated Tregs and identify genes controlled by c-Maf. We hope that our study will contribute to a better understanding of tissue-resident Treg origin and function both in health and disease.

This work is supported by a Fund for Research Training in Industry and Agriculture fellowship from the Fund for Scientific Research.

**WS.D1.01.02**

**Induction of intestinal Th22 cells by segmented filamentous bacteria**

U. Roy, E. Galvez, A. Gronow, M. Basii, A. Biehl, S. Huber, T. Strawig;

1 Helmholtz Centre for Infection Research, Braunschweig, Germany, 1 Medical University Hannover, Hannover, Germany, 1 University Hospital Hamburg-Eppendorf, Hamburg, Germany.

Heterogenous effector CD4+ T cells play an important role in modulating inflammation via their ability to produce distinct cytokines. Recently, Th22 cells have been identified as an important contributor of protection against enteropathogenic infection. While commensals inducing intestinal Th1, Th17 and Treg cells have been identified and extensively studied, Th22 cells have not been reported yet.

To assess the influence of microbiota composition to shape anti-bacterial IL-22 production, we utilized cytokine knock-in reporter mice (IL-22BFP x IL-17AGFP) colonized with different SPF and non-SPF communities as well as colonization with segmented filamentous bacteria (SFB). Early after enteric Salmonella Typhimurium (S. Typhimurium) CD4+ T cells and Treg cells that are secreting IL-17A have to be detected together with IL-22+ and SFB colonization is sufficient to induce Th22 cells and that they share a similar TCR V, µ4 enrichment than Th17 cells. Comparison of gene expression in IL-17A and IL-22 producing CD4+ T cells revealed significant differences between Th17 cells present in the steady state in the terminal ileum as well as Th22 and Th17 cells present during S. Typhimurium, such as the expression of Ifng and Il1β. In addition, we provide evidence that Th22 cells develop independently of IL-17 production using IL-17 fate mapping mice.

Together, our study identifies that SFB induces Th22 cells and that they differ from Th17 cells in their development and during infections in their gene expression profiles.

**WS.D1.01.03**

**Microbiota sensing by a MinC/lys x axis in dendritic cells promotes intestinal immune barrier via the steady-state regulation of IL-17 and IL-22**


1 CNIC, Madrid, Spain, 2 Kennedy Institute for Rheumatology, Oxford, United Kingdom, 3 University of Aberdeen, Aberdeen, United Kingdom, 4 Hospital Universitario de la Princesa, Madrid, Spain, 5 University of Zurich, Zurich, Switzerland.

Maintenance of the intestinal barrier keeps homeostatic host-microbiota relationship and depends on the cytokines IL-17 and IL-22. Production of these cytokines by type 1 T helper (Th17) cells and group 3 innate lymphoid cells (ILC3) is influenced by the gut microbiota in steady state. However, the host pathways that sense commensal microbiota homeostasis and instruct the appropriate responses need further investigation. We show that a microbiota-sensing pathway in dendritic cells (DCs) is critical for commensal-dependent production of intestinal IL-17 and IL-22. We identify MinC as a key cytokine receptor that detects mucosal-resident commensals and triggers IL-6 and IL-23 signalling, cytokines that regulate intestinal Th17 and ILC3 function. Absence of MinC or SYK in DCs impairs antimicrobial peptide and IgA production at the intestinal epithelium. Th17, sensing of commensals by MinC and SYK in DCs reinforces intestinal immune barrier and limits systemic microbial translocation, promoting host-microbiota mutualism.

**WS.D1.01.04**

**Control of RORγt regulatory T cell differentiation and intestinal immune homeostasis is dependent on the transcription factor c-Maf**


1 German Rheumatism Research Centre (DFRZ), Leibniz Association, Berlin, Germany, 2 Department of Cellular Immunology, Clinic for Rheumatology and Clinical Immunology, Charité - Universitätsmedizin, Berlin, Germany, 3 Molecular Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, 4 Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia, 5 Microbial Immune Regulation Research Group, Helmholtz Centre for Infection Research, Braunschweig, Germany, 6 Department of Medical Biology, University of Melbourne, Melbourne, Australia, 7 Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, 8 Department of Hematopoiesis, Sanquin Landsteiner Laboratory for Blood Cell Research, Amsterdam, Netherlands, 9 Department of Cancer Immunology, Genentech, San Francisco, United States.

Foxp3 regulatory T (Treg) cells are essential for immunological tolerance and homeostasis. In peripheral tissues, Treg cells acquire enhanced suppressive functions and co-opt regulatory T (Treg) cells are essential for immunological tolerance and homeostasis. In peripheral tissues, Treg cells acquire enhanced suppressive functions and co-opt function of multiple helper T cell subsets.

Th17 cells in the intestine. The increased Treg percentage suggests that the spontaneous colitis is a consequence of an altered Threg function rather than a quantitative defect.

Ablation of c-Maf in Tregs results in the development of spontaneous colitis, characterized by a rectal prolapse, increased Th17-associated cytokine expression and expansion of Th17 cells in the intestine. The increased Threg percentage suggests that the spontaneous colitis is a consequence of an altered Threg function rather than a quantitative defect.

**WS.D1.01.05**

**Il-17 receptor signaling in intestinal mucosal progenitor cells regulates epithelial cell regeneration**

P. Kumar, M. Beauxcore, K. Chang, A. Baneejee, T. Chu, H. Huang, X. Lin, S. Khalil, P. Joshi, A. Bialowas, V. Yang; Stanny Brook University, Brooklyn, United States.

Introduction: Il-17A, derived from Th17 cells, plays an important role in intestinal host defense. However, the interaction and potential molecular synergies between Il-17A and epithelial cell lineages, intestinal stem cells (ISCs), and progenitors of the intestine remain unclear. Materials and Methods: Il17ra<sup>-/-</sup> and Il17ra<sup>-/-</sup> mice were generated. A Dextran Sulfate Sodium (DSS)-mediated epithelial injury model was used. RT-PCR, immunofluorescence, and primary organoid cultures were used to study impact of Il-17A signaling on specific cell types. Results: Our data show that knockout of Il-17RA from the entire intestinal epithelium in Il17ra<sup>-/-</sup> mice are more susceptible to DSS-induced colitis. We found a defect in secretory goblet cell number in DSS-administered Il17ra<sup>-/-</sup> mice. Atonal homolog 1 (Atoh1) is a transcription factor required for intestinal secretory including goblet cell differentiation. Our preliminary data show a more severe DSS-induced colitis in Il17ra<sup>-/-</sup>;Atoh1<sup>cre</sup> mice, suggesting secretary progenitor or mature secretion epithelium-specific Il-17RA signaling regulates epithelial cell regeneration. UEA-1 and alcin blue stained colon tissues revealed reduced goblet cell number in Il17ra<sup>-/-</sup> mice. Next, we found reduced Sox9 expression as well as reduced K6/7<sup>+</sup> stained cells in the colon of Il17ra<sup>-/-</sup>;Atoh1<sup>cre</sup> mice. Furthermore, we show that Il-17A does not regulate mature goblet cell-specific functions. These data indicate a stem cell-specific defect. Based on this observation, organoids from Il17ra<sup>-/-</sup>;Atoh1<sup>cre</sup> mice were continuously stimulated with Il-17A. Continuous Il-17A stimulation lead to reduced orthogonal budding/morphogenesis, further confirming a Il-17A-mediated role in Atoh1-dependent epithelial cell regeneration. Conclusion Our data show a novel role of Il-17A in regulating Atoh1-dependent epithelial cell regeneration.
WORKSHOPS

WS.D1.01.06
Antigenic IgG augments intestinal inflammation in ulcerative colitis via IL-1 beta-dependent type 17 immunity
T. Castro-Dopico1, T. W. Dennison1, J. R. Ferdinand1, R. Matthews1, O. Cliff1, B. Stewart1, C. Lian1, K. Strangill1, E. Monk2, K. Saebo-Porsy1, C. E. Bryant4, M. Parker4, M. Clatworthy5, S. G. Howarth5, S. I. Gringhuis, T. B. Geijtenbeek2,1
1Molecular Immunology Unit, Department of Medicine, University of Cambridge, Cambridge, United Kingdom, 2MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, 3Division of Gastroenterology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom, 4Department of Surgery, University of Cambridge, Cambridge, United Kingdom, 5Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom.

Inflammatory bowel disease (IBD) is a chronic relapsing condition with two major subtypes, Crohn’s disease (CD) and ulcerative colitis (UC), both driven by aberrant immune responses. One of the strongest related findings in UC is with FCGR2A, the gene encoding an activating Fcγ receptor. FcγRs mediate the cellular effector functions of IgG. The UC-associated FcγRIIA-R131 variant is protective and reduces affinity for IgG. Since IgG is the dominant mucosal antibody, the role of IgG and FcγRs in IBD pathogenesis has been largely overlooked. Here we sought to investigate anti-commensal IgG in patients with UC and to determine the mechanism by which local IgG-FcγR engagement might contribute to intestinal inflammation in UC and murine models of dextran sodium sulfate (DSS)-induced colitis. We observed a significant increase in IgG-FcγRIIA-A/R131 expression and activated FcγR signalling and upregulated FCGR2A expression in IL-1β and CXC18, with hierarchical clustering confirming IL18 as the gene most closely associated with FCGR2A. Intestinal macrophages were the principle source of IL-1β and ex vivo stimulation with IgG immune complexes induced ROS-dependent 1IP production mediated by activation of the NLRP3 inflammasome. In vivo manipulation of the macrophage Fcγ activating/inhibitory ratio in mice determined IL-1β and Th17 cell induction. Finally, IL-1β blockade in mice with a high FcγR A/R ratio reduced IL-17 and IL-22-producing T cells and the severity of colitis. Our data reveal that commensal-specific IgG contributes to intestinal inflammation via FcγR-dependent, IL-1β-mediated Th17 activation.

WS.D1.02 Innate responses and immune signaling

WS.D1.02.01
The microbiota protects against respiratory infection via GM-CSF signaling
T. B. Clarke, R. L. Brown, R. P. Sequeira;
Imperial College London, London, United Kingdom.

The microbiota promotes resistance to respiratory infection, but the mechanistic basis for this is poorly understood. Here, we identify members of the microbiota that protect against respiratory infection by the major human pathogens Streptococcus pneumoniae and Klebsiella pneumojae. We show that the highest risk for respiratory infection in healthy adult mice is boosted when the vaccine is delivered 24 hours after adjuvant vaccine site preconditioning. Hence, we demonstrate a unique approach to facilitate optimal antigen delivery and specific antibody responses. Interestingly, when the vaccine is administered directly with the adjuvant, cytotoxic CD8

WS.D1.02.02
Hypercholesterolemia affects macrophage metabolism and function
J. Baardman1, M. van Weege1, S. Verberk2, K. H. Prange3, M. P. de Winter3, J. Van den Bosche3;
1Academic Medical Center, Amsterdam, Netherlands, 2VU University Medical Center, Amsterdam, Netherlands.

Metabolic reprogramming has emerged a crucial regulator of immune cell activation but how systemic metabolism influences immune cell metabolism and function remain to be investigated. Here we demonstrate that blood leukocytes from familial hypercholesterolemia (FH) patients show reduced expression of genes related to oxidative phosphorylation. To investigate the effect of dyslipidemia on immune cell metabolism, we performed in-depth transcriptional, metabolic and functional characterization of macrophages isolated from hypercholesterolemic mice. Systemicmetabolic changes in such mice translate into altered cellular macrophage metabolism and attenuates inflammatory macrophage responses.

WS.D1.02.03
MR1 recognition by monocytes and y6 T cells
J. Le Nours1, N. Gherardin1, S. Ramaratnam1, W. Awad1, B. Guly1, R. Berry1, F. Wieder2, D. Fairlie1, T. Tiganis3, J. McCluskey4, D. Pellicci1, A. Udrich1, A. Purcell4, D. Godfrey4, J. Rossjohn1,2,1;
1Biomedicine discovery institute, Monash university, Clayton, Australia, 2Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Australia, 3Peter Doherty Institute for Infection and Immunity, University of Melbourne, Parkville, Australia, 4Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Melbourne, Parkville, Australia, Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia, 4Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Queensland, Brisbane, Australia, 1Institute of Infection and Immunity, Cardiff University School of Medicine, Cardiff, United Kingdom.

The T lymphocytes repertoire is divided into two major lineages, q8 and y6 T cells, that are defined by their T cell receptor (TCR) gene-segment usage. The MHc-like molecule MR1 presents vitamin B derivatives to mucosal-associated invariant T cells (MAIT). Using MR1 tetramers, we characterized a population of MR1-restricted human y6 T cells that included phenotypically diverse Vb8-Vi1, Vb9-Vi3 and Vb8-Vi3 subsets, all of which exhibited MAIT autoactivity, independent on the nature of the bound ligand. The crystal structure of a y6TCR-MR1-antigen complex showed the y6TCR docked in a highly unusual manner that starkly contrasted all other TCR complex structures. The y6TCR bound under the MR1 antigen-binding cleft. Contacts were mediated largely by the TCR-δ-chain and more surprisingly by the α3-domain of MR1. Our findings reshape our understanding of TCR recognition determinants and y6 T cells.

WS.D1.02.04
RNA helicase DDX3 is a potent inducer of type I interferon and adaptive immunity
M. Stunnenberg1, S. I. Gringhuis, T. B. Geijtenbeek;
1Academic Medical Center, Amsterdam, Netherlands.

Strong innate and adaptive immune responses are paramount to prevent infection, but the genetic identity of the virus that triggers these responses is poorly defined. Here we have identified the RNA helicase human DEAD-box polypeptide 3 (DDX3) as a powerful pattern recognition receptor (PRR) to HIV-1 in dendritic cells (DCs). Here, we have investigated the capacity of DDX3 to induce potent innate and adaptive immune responses upon triggering with synthetic HIV-1-derived RNA ligands. DDX3 induces abortive HIV-1 RNA that is generated during HIV-1 transcription and encodes for the first 518-nucleotides of trans-activating protein Tat. Stimulation of DDX3 in DCs with synthetic abortive HIV-1 RNA induced INFα as well as IFN-stimulated genes such as TRIM5α and MxA. The type I IFN responses were functional as they strongly inhibited the replication capacity of HIV-1 in DCs. We next investigated whether DDX3 triggering with abortive RNAs also induces adaptive immune responses. DC treatment with abortive RNAs induced DC maturation and pro-inflammatory cytokine responses. DCs stimulated with abortive RNAs that were co-cultured with peripheral blood lymphocytes enhanced CD4+ and CD8+ T cell proliferation, thus supporting a role for DDX3 in inducing adaptive immunity. Comparison between putative viral ligands for DDX3 and other RIG-I-like receptors (RIGs) identified the abortive RNAs as the most potent triggers of immune responses. Abortive RNAs can be used as novel adjuvants for vaccine design and immunotherapy studies, since subsequent triggering of DDX3 evokes strong and functional antiviral immune responses. Thus, we have identified DDX3 as pattern recognition receptor of the RIG-I-like family by sensing HIV-1 and other viruses. AIdsfonds grant number P-8900.

WS.D1.02.05
Recruitment of DC-SIGN^+ monocyte-derived dendritic cells in the skin for antigen delivery and adaptive immunity
VU University Medical Center, Amsterdam, Netherlands.

Delivery of antigenic compounds to dendritic cells is a key to vaccination efficacy against tumors and pathogens. We have investigated the C-type lectin, DC-SIGN, as a target on dendritic cells to efficiently deliver antigens for the initiation of protective adaptive immune responses. Here, we show that subcutaneous injection of the adjuvant MF59 attracts DC-SIGN^+ antigen presenting cells to the skin and delivers vaccines. We identify the time-dependent influx of neutrophils and monocytes, including MoDCs, to the skin. Next, targeting DC-SIGN^+ skin-infiltrating cells with ovalbumin-coupled anti-mDC-SIGN antibody with adjuvant induces multifunctional antigen-specific CD8^+ T cells, as well as antigen-specific antibody responses. Interestingly, when the vaccine is administered directly with the adjuvant, cytotoxic CD8^+ T cell responses are preferred, whereas antibody production is boosted when the vaccine is delivered 24 hours after adjuvant vaccine site preconditioning. Hence, we demonstrate a unique approach to facilitate optimal antigen delivery and adaptive immunity shaped by a time-dependent sequence of vaccine events.
Enhanced trained immunity by targeting SHIP-1 in myeloid cells

P. Sar-Leal1, C. del Fresno2, P. Brandão3, S. Martinez-Cano3, O. M. Dungan3, J. D. Chisholm4, W. G. Kerr5, D. Sanchez1

1Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; 2Department of Chemistry, Syracuse University, Syracuse, NY 13210, United States; 3Department of Microbiology and Immunology, State University of New York (SUNY) Upstate Medical University, Syracuse, NY 13210, United States; 4Pediatrics Department, SUNY Upstate Medical University, Syracuse, NY 13210, United States.

Introduction: β-glucan-induced trained immunity in myeloid cells leads to long-term protective immunity against secondary infections through activation of the Dectin-1/PI3K (Phosphatidylinositol-3-Kinase) pathway. While previous studies have addressed the characterization of this phenomenon, strategies to boost trained immunity deserve further investigation. SHIP-1 is a hematopoietic-restricted inositol poly-phosphatase that limits PI3K activity and associates with Dectin-1. Thus, we wondered whether targeting SHIP-1 could modulate Dectin-1-mediated training.

Methods: Bone marrow-derived macrophages (BMDMs) from WT and LysM-Cre-SHIP-1 (LysMASHIP-1) mice were subjected to a β-glucan-induced trained immunity in vitro model. Receptor expression, cytokine production, pathway activation and metabolic status were analyzed. In vivo, both WT and LysMASHIP-1 mice were trained with β-glucan or Candida albicans and either with LPS to evaluate cytokine production or lethally infected with C. albicans to monitor protection. We also tested the effect of the SHIP-1 inhibitor 3AC (SHIPi) in our in vitro and in vivo models and in human peripheral blood mononuclear cells (PBMCs).

Results: β-glucan-trained SHIP-1-deficient macrophages enhanced TNFa production to a secondary LPS challenge, correlating with increased phosphorylation of Akt, targets of mTOR and elevated glycolytic metabolism. This enhanced trained TNFa production relied on epigenetic modifications. Trained LysMASHIP-1 mice showed increased TNFa production upon LPS challenge in vivo and better protection against reinfection with C. albicans. Pharmacological inhibition of SHIP-1 enhanced trained immunity against Candida infection, in mouse macrophages and human peripheral blood mononuclear cells.

Conclusions: Our data establish a proof of concept for trained immunity improvement and a strategy to achieve it by targeting SHIP-1.

WS.D1.03.02
Intestinal dysbiosis driven by dietary trp deprivation limits myelin-reactive T cell responses in a murine multiple sclerosis model

J. K. Sonner1, M. Keil1,5,1, M. Falk-Paulsen2, R. Bharti1, N. Mishra1, M. Kramer1, K. Deumelandt1, L. Wolf1, J. Deen2, T. V. Lan1,1,1,1, I. Oezen3,1, W. Wick2,1,1,1, P. Rosenstiel1, M. Plötzen1,1,1

1German Cancer Research Center, Heidelberg, Germany, 2Institute of Clinical Molecular Biology, Kiel, Germany, 3University Hospital Mainz, Mainz, Germany, 4Institute of Molecular Medicine, Mainz, Germany, 5University Hospital Heidelberg, Heidelberg, Germany, 6University Hospital Mannheim, Mannheim, Germany.

Multiple sclerosis (MS), the most common neurological disorder among young adults, is thought to be mainly driven by auto-reactive T cells that infiltrate the central nervous system (CNS). Recent data from preclinical studies suggest that these cells profoundly affect self-reactive T cell responses even in remote organs such as the CNS. The essential amino acid tryptophan (trp) and its metabolites have been identified as important modulators of local and systemic immune responses. While previous studies focused on trp as a source for endogenous aryl hydrocarbon receptor (AHR) ligands, we addressed the question whether dietary trp deprivation itself regulates myelin-reactive T cell responses using the murine experimental autoimmune encephalomyelitis (EAE) model. In this study we demonstrate that omission of protein, but also selectively trp from the diet protected from CNS autoimmunity. Dietary trp depletion did not prevent priming of myelin oligodendrocyte glycoprotein-reactive T cells, yet, attenuated encephalitogenicity of circulating T cells and prevented their infiltration into the CNS. Protection from EAE induction was independent of the host’s ability to sense trp via the stress kinase general control non-derepressible 2 (GCN2), but was critically dependent on the presence of the gut microbiome. Dietary trp deprivation caused mild, but evident intestinal inflammation, and, as revealed by 16S rDNA sequencing, induced substantial dysbiosis of the gut microbiome composition.

In summary, our data provide an important insight into the regulation of autoimmunity by dietary constituents and subsequent perturbations of gut microbiome homeostasis.

WS.D1.03.03
Gut dysbiota induces dendritic-cell traffic from colon to pancreatic lymph node: implications for activation of islet-reactive T cells in type 1 diabetes

R. Toivonen1, S. Siirola1,2, P. Pöysti1, E. Yarkan1, A. Tokoda1, M. Miyasaka1, A. Hänninen1

1University of Turku, Turku, Finland; 2University of Turku Central Animal Laboratory, Turku, Finland; 3Osaka University, Osaka, Japan; 4Turku University Central Hospital, Turku, Finland.

Studies in humans and mice suggest an important role for gut microbiota in the development of autoimmune type 1 diabetes (T1D). However, the exact mechanisms by which microbiota and gut immune system influence the development of autoimmune T1D remain elusive. Using KIKGR-reporter mice, we investigated the possible connection between the gut and pancreatic lymph nodes (PaLN) and the effects of dysbiosis on the traffic and activation of dendritic cells (DC) and T cells. We observed significant migration of dendritic cells (DC) from colon into colon-draining mesenteric lymph nodes (coMLN) but not to PaLN. A mild infection with Citrobacter rodentium also upregulated IFNy production by T cells. Migrated DC expressed XCR1, CD11b and CD103, allowing the same DC populations to appear also in PaLN, indicating that dysbiosis activates a route from colon to PaLN. C. rodentium also upregulated IFNy production by T cells. Migrated DC expressed XCR1, CD11b and CD103, typical for gut-derived and cross-presenting DC. Furthermore, in NOD mice C. rodentium accelerated infiltrates and upregulated TR2, TR8 and 4XCL10 expression by islet cells. Our results are in evidence for a direct, dysbiosis-activated route for DC and soluble material from the gut to the PaLN via lymphatics. This route may be the missing link between dysbiosis and its potential effects on T1D.

WS.D1.03.04
Imaging mass cytometry reveals the microanatomical location of B cell subsets in human gut associated lymphoid tissue

T. Tuff1, K. Todd2, N. Petrov2, R. Ellis2, S. Heck3,1, J. Spencer2

1Department of Microbiology and Immunology, State University of New York (SUNY) Upstate Medical University, Syracuse, NY 13210, United States; 2Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; 3European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands.

Human gut-associated lymphoid tissue (GALT) is important for host immune defence and for maintenance of homeostatic equilibrium throughout the gastrointestinal tract that is rich in antigens from the microbiota. Here we describe the use of imaging mass cytometry to delineate the microanatomical location of B cell subsets within GALT. Frozen sections from 3 human appendixes were stained simultaneously with 13 metal tagged antibodies and abated using a Fluidigm Hyperion™ imaging mass cytometer. Pixels were extracted from the image and clustered using Cytobank software to identify B cell subsets that were re-plotted onto the original image. Marginal Zone B cells in GALT (CD27+IgM+IgD+) were localized on the mucosal aspect of the germinal centre co-aligned with their CD27+IgD+IgM+CD45RB+ precursor population and sparse naïve B cells. Class switched memory B cells formed a separate zone from the marginal zone B cells on the periphery of the lymphoid tissue extending around the T cell zone and up to the follicle associated epithelium (FAE). These data showed that marginal zone B cells are present in the gut as in the spleen and that they are microanatomically separate from class switched memory B cells. IgM only B cells coalesced with both marginal zone and memory B cells consistent with data showing that they can be part of both populations. B cells in the FAE and cells expressing FcR like at the epithelial boundary were not of a single phenotype. This study demonstrates the power of imaging mass cytometry for accurate tissue mapping.

WS.D1.03.05
De novo fatty acid synthesis during mycobacterial infection is a prerequisite for the function of highly proliferative T cells, but not for dendritic cells or macrophages

L. Berod1, P. Stueve2, L. Minnari3, R. Bharti1, S. Hölscher1, L. Wolf3, S. Martínez-Cano4, J. D. Chisholm5, W. G. Kerr5, D. Sanchez1

1Department of Chemistry, Syracuse University, Syracuse, NY 13210, United States; 2Department of Immunology and Vaccine Center, Gothenburg University, Gothenburg, Sweden.

Mycobacterium tuberculosis (Mtb) is thought to interfere with macrophage lipid metabolism to ensure its persistence. In dendritic cells (DCs), fatty acid synthesis (FAS) has been suggested to permit optimal cytokine production and antigen presentation. We therefore determined the role of fatty acid metabolism in myeloid cells and T cells during BCG or Mtb infection, using genetic models allowing cell-specific deletion of acetyl-CoA carboxylase (ACC1) and 2 in DCs, macrophages or T cells. Our results demonstrate that FAS is induced in DCs and macrophages upon BCG infection. However, absence of ACC1 or ACC2 did not influence the ability of DCs and macrophages to cope with infection. In contrast, mice with a deletion of ACC1 specifically in T cells fail to generate efficient T cell responses and succumb early to Mtb infection. In summary, ACC1-dependent FAS is a crucial mechanism in T cells, but not DCs or macrophages, to fight mycobacterial infection.
Introduction

The role of death receptor signaling for pathogen control and infection-associated pathogenesis is multifaceted and controversial. Here, we show that during viral infection, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) modulates NK cell activity independently of its pro-apoptotic function. In mice infected with lymphocytic choriomeningitis virus (LCMV), TRAIL-deficiency led to improved specific CDB T cell responses, resulting in faster pathogen clearance and reduced liver pathology. Depletion experiments indicated that this effect was mediated by NK cells. Mechanistically, TRAIL restricts NK1.1-triggered IFNγ production by NK cells. In addition, TRAIL expressed by immune cells positively and dose-dependently modulates IL-15 signaling-induced granzyme B production in NK cells, leading to enhanced NK cell-mediated T cell killing. TRAIL also regulates the signaling downstream of IL-15 receptor in human NK cells.

The function of TRAIL on immune cells was so far confined to the induction of apoptosis on target cells. Our study reveals a hitherto unappreciated immunoregulatory role of TRAIL signaling on NK cells for the granzyme B-dependent elimination of antiviral T cells.

Objective

1. To investigate the role of TRAIL in NK cell-mediated immunity.
2. To determine the mechanisms underlying TRAIL-dependent NK cell activation.
3. To evaluate the impact of TRAIL on NK cell function during viral infection.

Methodology

Mice were infected with lymphocytic choriomeningitis virus (LCMV) or treated with TRAIL receptor antagonists. NK cell activation and function were assessed using flow cytometry and cytokine release assays. The role of TRAIL in NK cell-mediated immunity was evaluated in vivo using adoptive transfer experiments.

Results

TRAIL activation of NK cells primes memory-like cytotoxicity against cancer cells

CD16A activation of NK cells primes memory-like cytotoxicity against cancer cells

CD16A/FcγRIIA is a potent cytokine receptor on human NK cells, which can be exploited by therapeutic antibodies to target and eliminate cancer cells by NK cells. So far, the effects of CD16A-mediated activation on NK cell effector functions beyond classical antibody-dependent cytotoxicity have remained poorly elucidated. Here, we investigated how CD16A engagement of therapeutic antibodies greatly enhanced IL-2 and IL-15-driven NK cell proliferation and expansion.

References


CD56+ T cells were the main ILC3 population present, but numbers of these cells swiftly declined in the neonate and these cells were barely detectable in adult thymus.

Most prominent immediately after birth, but were rapidly diluted as the T cell development programme is increased. As observed in the embryonic thymus, CD45Rb+ lymphoid tissue inducer cells were the main ILC3 population present, but numbers of these cells swiftly declined in the neonate and these cells were barely detectable in adult thymus.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

97
This loss of ILC3 helps establish GATA-3"- ILC2 as the dominant ILC population in the thymus, with numbers of these cells gradually increasing during neonatal development. Innate lymphoid cells (ILCs) are a family of immune cells which can be subdivided into group 1, group 2 and group 3 ILCs according to the expression of signature transcription factors and cytokines. They share the same morphology as B and T lymphocytes; however, they lack rearranged antigen receptors. Data from human and mouse studies suggest that ILCs can influence the development and severity of graft-versus-host disease (GVHD), a common complication of allogeneic bone-marrow transplantation (BMT). Therefore, the definition of determinants of reconstitution and phenotype of ILCs post-BMT is a crucial first step to manipulating these cells for therapeutic benefit. Following lethal irradiation of CD45.2+BALB/c recipients and adoptive transfer of CD45.1+ C57BL/6 T cell depleted bone marrow, we found that recipient ILCs initially survived in non-lymphoid tissues post-irradiation but were slowly lost with time. Interestingly, despite full reconstitution of B and T lymphocytes by 60 days post-BMT, donor-derived ILCs remained severely depleted (11.8%, 7.7%, 11.6% of WT controls in small intestine, spleen and mLNs, respectively). In stark contrast, when performing BMT from BALB/c to C57BL/6, donor-derived ILCs were found to fully reconstitute in all tissues (small intestine=202.9%, spleen=151.6% and mLNs=235.9%) compared to WT controls (n=4). These data suggest that ILCs can reconstitute all non-lymphoid and lymphoid compartments following BMT however this may be dependent on a number of factors. We will discuss the potential role of the environment (microbiota - immune system interactions), strains and sex differences of BMT recipients all of which are currently being investigated by the laboratory.

**WS.D2.01.06**

**Killer cell Immunoglobulin-like receptor 3DL1 polymorphism defines distinct hierarchies of antigen recognition**

J. P. Vivian1, R. Saunders1, P. Pymm1, J. Rossjohn1, A. Brooks1; 1Monash University, Clayton, Australia, 2Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, The University of Melbourne., Melbourne, Australia.

The interaction between killer cell Immunoglobulin-like Receptor 3DL1 (KIR3DL1) and HLA-class I molecules has been linked to NK cell control of viral infections and malignancy. However due to the vast polymorphism of the genes encoding both its HLA-I ligands and the receptor itself, a mechanistic understanding of how these receptor/ligand interactions impact disease outcomes remains unclear. KIR3DL1 tetramers representative of the two major inhibitory KIR3DL1 lineages (*005 + *015) together with an interlineage recombinant (*001) were screened for reactivity against a comprehensive panel of HLA-I ligands. This revealed distinct hierarchies of preferred HLA-I ligands for each KIR3DL1 allotype, with KIR3DL1*005 recognising a wider array of HLA-I ligands than either the KIR3DL1*001 or *005. These differences in binding were also reflected in functional assays, utilising NK cell clones expressing specific KIR3DL1 allotypes. Intriguingly, while the polymorphic differences between KIR3DL1*001, *005 and *015 were remote from the KIR3DL1- HLA-I interface, the structures of the three KIR3DL1-HLA-I complexes showed that the broader specificity of KIR3DL1*005 correlated with an altered juxtapositioning of the D1-D2 domains and increased mobility within the ligand binding site, conferring a greater tolerance for disparate ligands. Collectively, we provide a molecular basis underlying the impact of KIR3DL1 polymorphism on HLA-I recognition that has important implications for haematopoietic transplantation. Reference: Saunders P. and Vivian J.P., et al Journal of Experimental Medicine. (2016) 213: 791-807.

**WS.D2.02 Molecular and cellular features of ILCs**

**WS.D2.02.01**

**Hobit specifically identifies ILC1 throughout peripheral tissues**

R. L. R. Tregonningbroek1, N. A. Kragten1, F. M. Behr2,1, L. Parga Vidal1, T. H. Wesseling1, R. Stark1,3, G. Gastgeiger1, K. P. van Gisbergen1; 1Erasmus Medical Center, Rotterdam, Netherlands, 2Department of Experimental Immunology AMC, Amsterdam, Netherlands, 3Institute of Systems Immunology, University of Würzburg, Würzburg, Germany.

Innate lymphoid cells (ILCs) form populations of lymphocytes involved in the regulation of inflammation, tissue repair, and immune homeostasis. These ILCs are present in mucosal barrier tissues such as the lamina propria and epithelium of the small intestine, the lungs and the salivary glands. Different subsets of ILCs have been recognized including ILC1, ILC2 and ILC3 that contribute to immune responses through the production of different sets of cytokines. The lack of ILC-specific tools has hampered the study of ILC development. Previously, we have shown that the transcription factor Homologue of Blimp-1 in T-cells (Hobit) regulates the maintenance of ILC1, but not of conventional NK cells in the murine liver. We now have developed Hobit Reporter mice, containing a “knock in” of the fluorescent protein tdTomato and CRE recombinase in the Hobit locus that allows us to specifically manipulate the expression of ILC1. We analyzed tdTomato expression in ILC subsets of the Hobit reporter mice and found high levels of Hobit expression in ILC1s in several peripheral organs including liver, small intestine and salivary gland. In contrast, conventional NK cells, ILC2 and ILC3 in these organs did not express Hobit. Using Hobit Cre x Red Stop (RSTOP) YFP lineage tracer mice, YFP expression was found in tdTomato+ ILC1, but not in tdTomato- NK cells, ILC2 and ILC3, suggesting that ILC1 do not contribute to the differentiation of other ILC subsets. We identify that Hobit defines ILC1 as terminally differentiated cells that are maintained as an independent population in the periphery.
Molecular definition of group 1 innate lymphoid cells in the mouse uterus

I. Filipovic1,2, L. Chiossone1, R. Vaccar1, R. S. Hamilton3, J. Doisne2, A. Sharkey2, C. Mignan2, L. Moretta2, F. Ciccoli1,2
1Centre for Translational Research, University of Cambridge, Cambridge, United Kingdom, 2Department of Obstetrics and Gynaecology, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom, 3Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom, 4G. Gaslini Institute, Genoa, Italy, 5Department of Experimental Medicine (DIMIES), University of Genoa, Genoa, Italy, 6Department of Immunology, Pasteur Institute, Paris, France, 7Department of Pathology, University of Cambridge, Cambridge, United Kingdom, 8Department of Experimental Medicine (DIMIES), University of Genoa, Genoa, Italy, 9Department of Immunology, IRCSS Bambino Gesu Children’s Hospital, Rome, Italy.

Determining the function of uterine lymphocytes is challenging because of the rapidly changing nature of the organ in response to sex hormones and, during pregnancy, to the invading fetal trophoblast cells. Here we provide the first genome-wide transcriptome atlas of mouse uterine group 1 innate lymphoid cells (g1 ILCs) at mid-gestation. The subset composition of g1 ILCs fluctuates throughout reproductive life, with Eomes+CD49a+ cells dominating before puberty and specifically expanding in second pregnancies, when the expression of CXCR6, a marker associated with memory NK cells, is upregulated. Tissue-resident Eomes+CD49a+ NK cells (trNK), which resemble human uterine NK cells, are most abundant during early pregnancy, and showcase gene signatures of responsiveness to TGFB and IL-1, and connections with trophoblast, epithelial, endothelial and smooth muscle cells, leucocytes, as well as extracellular matrix. Tissue-resident NK cells express genes involved in aerobic glycolysis, lipid metabolism, iron transport, protein ubiquitination, and recognition of microbial molecular patterns. Conventional NK cells expand late in gestation and may engage in crosstalk with trNK cells involving IL-18 and IFNγ. These results identify trNK cells as the cellular hub of uterine g1 ILCs at mid-gestation and mark Eomes+CD49a+CXCR6+ g1 ILCs as potential memory cells of pregnancy. This work is supported by grants from the Wellcome Trust and Centre for Trophoblast Research for Fertility.

WS.D2.02.06

NCR+ ILC3 maintain larger STAT4 reservoir via T-BET to regulate type 1 features upon IL-23 stimulation in mice.

Y. Mikami1, G. Scarno1, B. Zhitn1, H. Shih2, Y. Kanem1, A. Santoni1, J. I. O’Shea1, G. Sciume1
1National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD, United States, 2Sapienza, University of Rome, Rome, Italy, 3HERM, Department of Medicine, Karolinska Institutet, Stockholm, Sweden.

Type 3 innate lymphoid cells (ILC3) represent a heterogeneous group of cells producing interleukin(IL)-22 and/or IL-17, and involved in regulation of gut barrier homeostasis and inflammation. Cytokines activating the Janus kinases (Jaks) and members of the signal transducer and activator of transcription (STAT) pathway are key players in lymphoid development, differentiation and activation. In this context, we have previously dissected the role of STAT4 in regulation of ILC development in mice and revealed that ILC3 expressing natural cytotoxicity receptors (NCR+ ILC3) are particularly sensitive to deprivation of STAT4 signals. Here, by using mouse models in combination with genetic and transcriptomic approaches, we defined at the molecular level, initial events of NCR+ ILC3 activation and the contribution of STAT4 in the acquisition of type 1 features, downstream of IL-23 stimulation. In particular, we observed high basal expression of STAT4 in NCR+ ILC3, dependent on T-BET, which contributes to enhance production of IFNγ. Altogether, our findings shed light on a feed-forward mechanism involving STAT4 and T-BET that modulates the outcome of IL-23 signaling in ILC3.

WS.D3.01 Novel vaccine approaches to intracellular pathogens

Targeted delivery of BCG vaccine to the DEC-205 receptor improves protective efficacy against Mycobacterium tuberculosis

C. Counoupas1, R. Pinto1, L. Baker2, G. Ngalingam1, A. Niso3, W. Britton3, J. Triccas3
1Centenary Institute, Sydney, Australia, 2University of Sydney, Sydney, Australia.

Tuberculosis (TB) remains a major cause of mortality and morbidity worldwide, with 1.7 million deaths annually and a third of the world infected with the latent form of the disease. The currently available vaccine, M. bovis BCG, is only partially effective against TB, and new strategies are required to control the global epidemic. We hypothesised that BCG may interact sub-optimally with dendritic cells (DCs), the cell type pivotal in shaping the adaptive immune response to infectious agents. Thus strengthening this interaction could improve BCG protective efficacy. In order to do this, we engineered BCG to express the single-chain variable fragment (ScFv) recognizing the type-I lectin receptor DEC205 (BCG-DEC), which is expressed predominantly on migratory DCs. An increased functional ability of BCG-DEC to interact with DEC205-expressing cell lines and bone marrow derived DCs was observed; interestingly, the increased interaction resulted in an augmented secretion of inflammatory cytokines and chemokines by BMDCs. Given the high levels of expression of DEC205 in the skin of mice, we tested the protective efficacy of BCG-DEC after subcutaneous vaccination. We found that BCG-DEC conferred greater protection than BCG 4 weeks after aerosol challenge with virulent M. tuberculosis, and its efficacy lasted up to 20 weeks post infection, even when BCG protection had waned. Our results indicate that targeting DCs by modifying BCG is an effective strategy to improve protection against TB.
During experimental tuberculosis, interleukin-27 regulates protection and limits the expansion of multifunctional T cells by inhibiting interleukin-17A

K. Ritter1, J. Behrends1, A. Hälscher1, J. Volf1, I. Rosenkranz2, H. Erdmann3, C. Hälscher1;
1Infection Immunology, Research Center Borstel, Borstel, Germany, 2Fluorescence Cytometry Core Unit, Research Center Borstel, Borstel, Germany, 3Department of Infectious Disease Immunology, Statens Serum Institut, Copenhagen, Denmark.

After infection with Mycobacterium tuberculosis (Mtbb), mice lacking the IL-27R exhibit lower bacterial burdens but develop immunopathological sequelae in comparison to wildtype mice. This phenotype correlates with an enhanced recruitment of antigen-specific CD6+CD4+ T cells and an increased frequency of IL-17A-producing CD4+ T cells. By contrast, when mice are infected in C57Bl/6, IL-27R-deficient and IL-27R/IL-17A-deficient mice, we observed that in the absence of IL-27R, IL-17A increased protection and elevated interleukin-17A is supported by IL-27A. Whereas IL-27A does neither impact the development of Th1 cells nor the expression of PD1 and IL6/IL8 on T cells if IL-27R-deficient mice during infection, it significantly regulates the presence of multifunctional T-cells, co-expressing IFN-γ, IL-2 and TNF, and highly stratified granulomas in the lungs. In addition, IL-17A supports Cx3c, Cx41D and Cx13 expression in lungs of infected IL-27R-deficient mice. Taken together, IL-17A contributes to protection in Mtbb-infected IL-27R-deficient mice probably through a chemokine-mediated recruitment and strategic positioning of multifunctional T cells in granulomas. Although IL-27 also prevents IL-17A-mediated immunopathology, a timely inhibition of IL-27R-mediated signaling during vaccination or host-directed therapy of tuberculosis might increase a coordinated protective effect of IL-17A without detrimental consequences.

Human Plasmodium falciparum infection induces a predictable and persistent distortion in transcriptomic landscapes across T cell subsets

T. S. Watkins1,2, E. Amante1, S. Turner1, S. Darka1, A. Ronier1, D. L. Doolan1, D. C. Douek2, S. J. McCarthy2, J. J. Miles1,2,3;
1ATHM, James Cook University, Cairns, Australia, 2QIMR Berghofer, Brisbane, Australia, 3University of Queensland, Brisbane, Australia, 4VRC, NIH, Bethesda, United States.

Malaria infection still remains one of the world’s most formidable issues across both health and economical systems. Despite the best intervention strategies over 200 million people are infected annually, and mortality rates exceed 400,000 deaths per year. To develop effective therapeutics and vaccines a better understanding of fundamental human immunology is required. This is particularly true of the contribution of T cells to malaria defence. Research to date comes from field samples which can sometimes be difficult to interpret due to confounding factors including multiple exposures. As such, the underlying immune correlates of an efficient response remain unknown. To investigate the role of T cells in disease we used a human model of blood stage Plasmodium falciparum infection involving the Medicines for Malaria Venture (MMV). We performed high-dimensional phenotyping and T cell receptor (TCR) sequencing of T cell subsets before infection, during infection and during convalescence. Plasmodium falciparum infection resulted in marked phenotypic ‘scaring’ across all T cell subsets and persisted during convalescence. We identified gene modules that correlated with parasitemia, revealing a possible continuum between individuals at risk of disease and ‘elite controllers’. Remodelling of the TCR repertoire could also be correlated with transplantation changes, and gene expression and TCR repertoire metrics could serve as novel proxies of disease burden and assist in identifying pathways for immunotherapies or vaccine activation. Indeed, characterizing the T cell biology of elite controllers opens the possibility of inducing this phenotype for the purposes of rational vaccine design and therapeutic intervention.

BCG-induced trained innate immunity during controlled human malaria infection

J. Walk1, C. de Bre1, W. Graumann1, R. Siebelink-Stoter2, G. van Gemert1, M. van de Vegt-Bolmer1, K. Teelen1, C. H. Ehrnshein1, R. J. Arts1, M. C. Behet1, S. J. Moolla1, A. Yang1, R. van Crevel1, P. Adly1, G. de Maat1, A. van der Jen1, C. Isabell Benn1, M. Netea1, R. W. Sauerwein1;
1Radboud university medical center, Nijmegen, Netherlands, 2Statens Serum Institut, Copenhagen, Denmark.

Recent evidence suggests that certain vaccines, including Bacillus-Calmette Guérin (BCG), can induce changes in the innate immune system with non-specific memory characteristics, termed ‘trained immunity’. We performed a randomized, controlled clinical trial in twenty healthy male and female volunteers to evaluate the induction of immune and protective efficacy of BCG vaccination against a controlled human malaria infection (CHMI) five weeks after vaccination. BCG vaccinated volunteers had earlier and more severe clinical symptoms, and showed a heterologous, memory-like monocyte and (in)active lymphocyte re-activation that correlated with reduced parasitemia. These findings demonstrate for the first time that BCG vaccination induces trained immunity with functional activity against an unrelated, clinically relevant, human pathogen in vivo. It forms a strong impetus to further explore its potential in the clinical development of a rational malaria vaccine strategy.

Evaluation of multi-epitope peptides derived from different Leishmania species as potential vaccine candidates against human Leishmaniasis

S. Hamrouni1,2, A. Kidar1, R. Chamakh Ayori1, E. Pettididier1, J. Lemese1, R. Bras Gonçalves1, A. Meddeb Garnaou1;
1Institut Pasteur, Tunis, Belvédère, Tunisie, 2Hôpital régional, Gafsa, Tunisie, 3Institut de Recherche pour le Développement, Montpellier, France.

IFN-γ-producing-Th1 cells are required for parasite control. Healing is correlated with resistance to reinfection, suggesting that vaccination is feasible. To date no vaccine is available.

To identify peptide vaccine candidates against human leishmaniasis, we evaluated the immunogenicity of multi-epitope peptides in individuals with healed cutaneous leishmaniasis due to Leishmania major.

PSA (Promastigote Surface Antigen), LmrRAB (L. major large RAB GTPase) and H2B (Histone), reported to be immunogenic in humans, mice and dogs, were screened in silico for T cell epitopes able to bind to frequent HLA class-I and -II alleles. Selected peptides were synthesized, pooled and used to screen peripheral blood mononuclear cells from subjects cured of cutaneous Leishmaniasis. Healthy subjects were used as control group. IFN-γ and IL-10 production was evaluated by ELISA. Phenotypes of cytokines producing T cells were analyzed by flow cytometry.

We showed that some peptide pools were able to elicit significant IFN-γ levels in cured but not in healthy individuals. No IL-10 production was detected. A significant increase in the percentage of IFN-γ-producing CD4+ T cells and polyclonal CD4+T cells producing IFN-γ, TNF-α and IL-2 was detected in response to peptide pools. A significant increase of the percentage of CD4+CD45RO+CCR7+central memory T cells was also observed. In conclusion, we demonstrated that multi-epitope peptides derived from different Leishmania species could be used to induce IFN-γ and polyclonal CD4+T cells, both associated with protection against Leishmania infection, suggesting that they may be exploited as vaccine candidates.

NIHV Travel Grants for Early Career Scientists from Developing Countries

Novel vaccine approaches for viruses

Deciphering the complexity of vaccine-induced immunity with omics technologies: innate immune responses differ between priming and boosting immunization

J. L. Palgen1, N. Tischteich1,2, J. Elmhousen-Voones1, S. Delandre1, I. Namet1, N. Huot1, P. Rosenbaum1, N. Dereudeur-Bosquet1, F. Martinon1, A. Cosma1, M. Müller-Trutwin1, Y. Levy1, R. Le Grand2, A. S. Beignon2;
1CEA – Université Paris Sud 11 – INSERM U1184, Immunology of Viral Infections and Autoimmune Diseases, IDMIT department, IBFJ, Fontenay-aux-Roses, France.

A better understanding of vaccine-induced innate responses and their impact on immune memory is critical to more efficiently design long-term protective vaccines, but the immune system complexly adapts itself making it challenging. To take up this challenge, omics technologies are promising since they allow multiparametric and multiscale measurements.

Here, we used mass cytometry and DNA micro-arrays to longitudinally study blood innate cells (granulocytes, monocytes, DC and NK cells) during prime-boost vaccination of cynomolgus macaques with the Modified Vaccinia virus Ankara (MVA) attenuated vaccine. We developed new analyses pipelines to explore those datasets and assess the magnitude, quality and dynamics of immune responses after each immunization.

Innate myeloid and lymphoid cells numbers were similarly early, transiently and strongly impacted after each immunization. However, at a deeper resolution, several subphenotypes were differently enriched/attributed between each immunization. The key features of prime-boost differences, identified using multivariate analyses, include the differential expression of CD11b, C66D, CD45 and CD132 on neutrophils; CCR5, CXCR4, CD45 and CD16 on cDCs; CD81, CD11a, CD14 and CD45 on monocytes; and CD16, CCR5, CD69 and CD107a on NK cells. More strikingly, before the boost, the subphenotype composition of innate cells differed from baseline with the upregulation of those activation/ inflammation markers, strongly suggesting that the innate immune system is imprinted after the prime.

This work indicates that, similarly to adaptive responses, innate responses differ after each vaccine encounter. This revisits vaccine-induced innate responses and open new perspectives with the involvement of innate training/memory.
WS.D3.02.02
Identification of vaccine responding T-cells using TCR repertoire sequencing data

M. V. Porogorets1,2, A. A. Minervina1, M. Puelma Touzel3, A. E. Komech1, E. I. Kovalenko1, G. G. Karganova1, E. S. Egorov1, A. Y. Komkov1, D. M. Chudakov1, I. Z. Mamedov1, T. Mora1, A. M. Walczak1, Y. B. Lebedev1.
1Shemyakin-Ovchinnikov Institute of Bioorganic chemistry RAS, Moscow, Russia; 2Research Institute of President of RF for Life Science, Moscow, Russia; 3Laboratoire de physique théorique de l’École Normale Supérieure, PSL University, CNRS, Sorbonne Universités, UPMC, Paris, France; 4Chumakov Institute of Poliomyelitis and Viral and Rickettsial Diseases, Moscow, Russia; 5Laboratoire de physique théorique, CNRS, Sorbonne Université, Université Paris-Diderot, et École normale supérieure (PSL), Paris, France.

Introduction: High-throughput sequencing allows for deep profiling of TCR repertoires, however our ability to extract clinically relevant information from this data is limited. We develop approaches to identify clonotypes participating in immune response from both longitudinal repertoire data and single snapshot of repertoire.

Methods: We collected PBMCs, CD4+, CD8+ T-cells at 5 timepoints before and after yellow fever immunization of 3 pairs of twins. We sequenced TCR repertoires from all samples on illumina HiSeqs.

Results: Using a novel statistical approach, we identified 500-1500 clones expanded at the peak of the response (day 15) in each individual, occupying up to 5% and 8% of CD4+ and CD8+ repertoires. We validated these clonotypes by an IFN-γamma secretion assay, MHC-multimer staining and sorting of activated T-cell subpopulations. We found little sequence overlap even between YF-reactive repertoires of identical twins, but lots of YF-reactive TCR sequences in different donors were homologous. We also developed an algorithm to identify clusters of similar TCR VD-J types in single repertoire samples. Such clusters were almost absent before immunization and very abundant at the peak of the response, where they mostly consisted of YF-specific sequences (40%-75%). We also found such clusters (immune response signatures) in published immunotherapy and autoimmune repertoire sequencing data.

Conclusions: T-cell response to YF-vaccine is strong and almost unique in identical twins. Highly homologous sequences in antigen-specific repertoires allow us to build an algorithm to identify clusters of similar clonotypes participating in an active immune response in single repertoire samples. Supported by RSF-15-15-00178

WS.D3.02.03
Mucosal vaccine mediates cross-protection by immune training

P. Brandi1, L. Conejero1,2, S. Cuesto1, S. Martinez-Canor1, P. Szcz-Leal1, M. Enamorado1, J. Amores-Iniesta1, J. Subiat1, D. Sancho1.
1Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; 2Inmunotec S.L., Madrid, Spain.

Respiratory tract infections by viruses and bacteria are a leading cause of morbidity worldwide. MV130 is a mucosal vaccine that consists of whole inactivated bacteria that are common respiratory pathogens. It induces the frequency and severity of infectious episodes in patients with recurrent respiratory tract infections, including episodes of virus-induced wheezing in children, a significant risk factor for asthma in later life. However, the mechanisms by which MV130 controls these episodes are still poorly understood.

We now show that immunization with pICLC-adjuvanted Gag protein also induced T helper cell responses sufficient to provide intrastructural help and to significantly reduce the frequency of respiratory tract infections in patients with recurrent respiratory tract infections, including episodes of virus-induced wheezing in children, a significant risk factor for asthma in later life. Moreover, their mechanism is not related to the immunogenicity of the vaccine, as it is also observed in patients with low levels of antibody response.

Conclusions: Intrastructural help (ISH) is an interesting strategy to enhance overall levels of HIV Env antibodies, to modulate their IgG subtype ratio, and to increase T helper cell responses involved in control of HIV infection.

WS.D3.02.04
Liver-resident memory CD T cells develop during chronic viral infection, have reduced effector functions but can be reactivated to clear persistent viral infection of hepatocytes

Institute of Molecular Immunology, Munich, Germany.

Introduction: Hepatic tissue-resident memory (TRM) CD T cells are found after acute and self-limited viral infections. It is unclear whether liver T RM are also found during chronic infections.

iMATEs (intrahepatic-myeloid-cell-aggregates-associated-with-T-cell-expansion) that serve as hubs for T cell-expansion to promote T-cell immunity. We characterized the impact of chronic infection and iMATE induction for liver T RM function and viral infection control, respectively.

Methods: Infection with liver-targeting adenoviruses encoding ovalbumin under CMV promoter (Ad-CMV-GOL) or hepatocyte-specific TPR promoter (Ad-TR-GOL) caused acute self-limited or persistent liver-infection, respectively. 100 CD45-1 iMATEs (intrahepatic-myeloid-cell-aggregates-associated-with-T-cell-expansion) that serve as hubs for T cell-expansion to promote T-cell immunity. We characterized the impact of chronic infection and iMATE induction for liver T RM function and viral infection control, respectively.

Results: CXCR6+ liver T RM developed after self-limited Ad-CMV-GOL but also after chronic Ad-TR-GOL liver infection and were not clonal deleted. Compared to self-limited infection, no CXCR3+ expressing antigen-specific hepatic T cells were detected during chronic Ad-TG-GOL infection. At 90d after chronic Ad-TG-GOL infection, liver T RM showed severely impaired cytotoxic-capacity and cytokine-production, thus revealing functional adaption to persistent infection. RNA-sequencing of liver T RM from acute-self-limited chronic infection revealed chronic inflammation and fibroinflammatory expansion to identify exhausted lung T RM and found a single exhaustion-specific transcription factor. Importantly, iMATE-induction led to increased effector function of hepatic virus-specific T-cells and control of persistent infection.

Conclusions: Exhausted liver T RM are formed during chronic viral-infection and are identified using novel biomarkers. iMATE induction overcomes liver T RM dysfunction to control chronic infection. Thus, our results provide novel markers for immunomonitoring of patients with chronic viral hepatitis and demonstrate functional plasticity of exhausted liver T RM local hepatic stimulation within iMATEs.

WS.D3.02.05
Harnessing T-helper (Th) cell responses induced by licensed vaccines for HIV vaccine development

1University Hospital Erlangen, Institute of Clinical and Molecular Virology, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany; 2National Research Center, Genetic Engineering and Biotechnology Division, Department of Microbial Biotechnology, Giza, Egypt; 3Department of Molecular and Medical Virology, Ruhr-University Bochum, Bochum, Germany; 4CSIRO Materials Science and Engineering, Parkville, Victoria, Australia.

Strong T-helper responses could significantly improve antibody-based effector mechanisms induced by HIV vaccines. We recently reported that Gag-specific Th responses induced by gene-based vaccines improved the quality of the Env-specific antibody in mice after booster immunizations with virus-like particle (VLP) containing Gag and Env via intrastructural help (ISH). We now show, that immunization with plCl-adjuvanted Gag protein also induced T helper cell responses sufficient to provide ISH and to significantly increase Env-specific IgG2a levels after VLP booster immunizations. We therefore evaluated whether T helper cells induced by the licensed Tandempur or HBVAPX-Pro vaccines could be harnessed to improve the HIV-1 Env-specific antibody response. To this end, we generated HIV-VLPs incorporating Th epitopes for tetanus-toxoid (TT) and HBsAg (HB) within Gag. After immunizing Tandempur-primed mice with VLPs containing the TT-Gag, Env-specific IgG1 antibody levels increased up to 100-fold compared to non-vaccinated mice. The avidity of the HIV-Env antibodies was also higher. HBVAPX-Pro-primed mice boosted with VLPs containing HB-Gag showed an approximately 10-fold increase in Env-specific IgG1 levels. Priming mice with DNA vaccines encoding TT, HBsAg or Gag predominantly improved Env-specific IgG2a responses after booster immunization with epitope-matched VLPs. However, if VLPs lacked matched epitopes, the priming immunization had no effect on the HIV-1 Env-specific antibody response. Thus, harnessing T helper cells induced by licensed vaccine to provide intrastructural help for HIV-1 Env-specific B cells is an interesting strategy to enhance overall levels of HIV Env antibodies, to modulate their Ig subtype ratio, and to increase their avidity.

WS.D3.02.06
Design of TLR2 ligand-synthetic long peptide conjugates for therapeutic vaccination of chronic HBV patients

Y. Dau1, D. T. S. Jansen1, A. van den Bosch1, R. A. de Man1, N. van Manfoort1, G. G. Zorn1, W. Krebber1, C. J. Melief1, A. M. Wortman1, S. I. Buschow1.
1Department of Gastroenterology and Hepatology, Erasmus Medical Center Rotterdam, Rotterdam, Netherlands, 2ISA Pharmaceuticals BV, Leiden, Netherlands.

Synthetic long peptide (SLP) vaccination is a promising new treatment strategy for patients with a chronic hepatitis B virus (HBV) infection. Previously, we have shown that a polyclonal B cell receptor derived SLP is capable of boosting CD4+ and CD8+ T cell responses in patients ex vivo. These T cell responses were further enhanced by adjuvants like TLR2 ligands. For optimal effect of a therapeutic vaccine adjuvants can be conjugated to the SLP to ensure uptake by the same cell and thus presentation of SLP-contained epitopes by mature dendritic cells (DC) only. Here, we evaluated the efficacy of TLR2-ligand conjugated SLPs of different lengths and by different conjugation strategies. Engineered HBV-specific CD8+ T cells were used to evaluate the cross-presentation of conjugates by in vitro-generated and naturally-occurring DC subsets. Results indicated better T cell responses were induced by a shorter (16 amino acids) SLP conjugate compared to longer (26 and 37 amino acids) conjugates indicating size may hamper cross presentation.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 101
LONGER SLPS however are, preferred as inclusion of multiple CD4 and CD8 epitopes is required to ensure population-wide and long-lasting responses. To reduce size effect, we determined the number of pathogen-specific epitopes in which a cationic-salt in which a cationic-salt was placed between the TLR2 ligand and the long SL, to facilitate an endosomal release. We found this linker improved cross-presentation, affected SLP intracellular processing and overall more effectively triggered patients' T cell responses ex vivo. These results provide an impetus step forward in the design of a therapeutically SLP-based vaccine to cure chronic HBV.

WORKSHOPS

WS.D4.01 Protective mechanisms for microbial pathogens

WS.D4.01.01

Different composition and functional properties of splenic T cells in experimental cerebral malaria-susceptible C57BL/6 wildtype and protected fnaro+ mice

J. J. Reichwald1,2, P. J. Korir1, L. Jenster1, A. Mueller1, A. Hoerauf1, B. Schumak1

1Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany; 2Centre for Infectious Diseases, Parasitology Unit, Heidelberg University Hospital, Heidelberg, Germany

Introduction: Cerebral malaria (CM) is a severe complication of Plasmodium infection and can be studied experimentally in Plasmodium berghei ANKA (Pba-)infected C57BL/6 wildtype (WT) mice that develop experimental CM (ECM). Pba-infected interferon (IFN) alpha receptor knock out (fnaro+ ) mice are protected from ECM. We characterized the composition and activity of splenic T cells between ECM-susceptible WT mice and ECM-protected mice and analyzed whether these cells have different functional properties.

Material and Methods: C57BL/6 WT and fnaro+ mice were infected with Pba. 6 days post infection (dpi) splenocytes were analysed by flow cytometry, cytokine/chemokine levels were determined in supernatants of splenocyte cultures with enzyme-linked immunosorbent assay (ELISA) and with intracellular FACS.

Results: ECM-positive WT mice contained on dpi 6 CD8 T cells in their brains while presenting decreased numbers of splenic CD8+ T effector cells and memory cells. Pba-infected ECM-protected ko mice lacked T cell infiltration in their brains while presented higher numbers of splenic CD8+ effector T cells and CD4+ effector memory T cells. Whereas IFN-y levels were comparable in splenocyte cultures of both mouse groups, WT mice contained less CCL5 in their spleens upon infection than protected mice. T cells of all Pba-groups produced more granzyme B and CCL5 than naive controls. Conclusions: CD8+ T cells and memory cells were generally retained in the spleen of Pba-infected ECM-protected mice and might miss an egress signal migrating to the brain. Funding: Jurgen-Manacht-Stiftung (PHD scholarship J.R.J.), BONFOR (B.S.), ECX1023 (A.H., B.S., J.R.J.)

WS.D4.01.02

A distinct subset of human CD56+ cells present at baseline in HBaS-SCT-children is associated with a lower parasite density during an episode of clinical malaria

C. Loiseau1,2, D. K. Dumba1, B. Traore1, J. L. Brody1, C. Pirojfi1, P. D. Crompton1, D. L. Doolan1

1Australian Institute of Tropical Health and Medicine, Cairns, Australia; 2James Cook University, Cairns, Australia; 3University of Sciences, Technique, and Technology of Bamako, Bamako, Mali; 4National Institutes of Health, Rockville, United States

Despite mounting substantial immune response, humans fail to control Plasmodium spp. infection. However, some people display better immune responses and parasite control; among them are individuals presenting the sickle-cell trait phenotype which corresponds to the heterogeneous state of haemoglobin A and S (HBaS-SCT). Here, we compared the immune profiles of HBaS and HBaS-SCT Malian children from a malaria-endemic area, to define immunological differences that could potentially explain the relative protection observed in HBaS-SCT-patients.

Blood samples were obtained from HBaS-SCT- and HBaS-children at their uninfect ed baseline and during their first febrile malaria episode of the ensuing malaria season. PBMCs were isolated, activated, and multicolor flow cytometry analysis were performed. Functional assays were performed on PBMCs from healthy donors.

At baseline, the frequency of CD56+ cells was increased in HBaS-SCT-children (P<0.05), and was inversely correlated with parasite density (r=-0.76, P<0.05). The CD38hi subset in CD56+ cells was reduced in HBaS-SCT-children (P<0.01); and was correlated with parasite density (r=-0.90, P<0.01). Thus, the increased frequency of the CD56+ cells in CD3+ lymphocytes of HBaS-SCT-children appeared to be due to their CD38+subset. This subset of NK cells was inversely correlated with parasite density (r=-0.86, P<0.05), characterized by an increased expression of HLA-DR and CD45RO (P=0.001) and a 15-fold increased production of IFN-γ compared to the CD38lo subset. This CD38+subset represents a novel population of CD56+ cells identified at baseline in HBaS-SCT-children and is potentially associated with enhanced parasite control. The HLA-DR+CD45RO+CD38hiCD56+CD3 cells may represent a potential predictive signature of immunity to malaria.

WS.D4.01.03

Transcription factor T-bet plays a complex role in B cell mediated immune response against Plasmodium infection

M. Akkaya1, P. W. Sheehan1, C. K. Cimperman1, B. P. Theall3, M. Pena1, S. K. Pierce1

1National Institutes of Health, Rockville, United States

Malaria is a global health concern which affects over 200 million individuals worldwide. Although immune system rapidly responds to Plasmodium infection with specific antibodies, natural antibodies fail to establish long term protection, often leading to repetitive infections and chronicity. Recent studies showed that in chronic malaria setting both B cells and T-bet may not be equally crucial for all B cell responses to infection. However in vitro stimulation of WT and T-bet KO B cells with Anti-IgM plus IFN-gamma reveals no differences in IFN-gamma production or CD38 expression in CD56low/med+ T cells and memory cells were generally retained in the spleen of PbA-infected ECM-protected mice and might miss an egress signal migrating to the brain.

Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany; 2Centre for Infectious Diseases, Parasitology Unit, Heidelberg University Hospital, Heidelberg, Germany; 3German Centre for Infection Research (DZIF), partner site Bonn-Cologne, Germany

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Material and Methods: C57BL/6 WT and fnaro+ mice were infected with Pba. 6 days post infection (dpi) splenocytes were analysed by flow cytometry, cytokine/chemokine levels were determined in supernatants of splenocyte cultures with enzyme-linked immunosorbent assay (ELISA) and with intracellular FACS.

Results: ECM-positive WT mice contained on dpi 6 CD8 T cells in their brains while presenting decreased numbers of splenic CD8+ T effector cells and memory cells. Pba-infected ECM-protected ko mice lacked T cell infiltration in their brains while presented higher numbers of splenic CD8+ effector T cells and CD4+ effector memory T cells. Whereas IFN-y levels were comparable in splenocyte cultures of both mouse groups, WT mice contained less CCL5 in their spleens upon infection than protected mice. T cells of all Pba-groups produced more granzyme B and CCL5 than naive controls. Conclusions: CD8+ T cells and memory cells were generally retained in the spleen of Pba-infected ECM-protected mice and might miss an egress signal migrating to the brain.

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Within host-adaptation of Bordetella pertussis under vaccine pressure

E. van Schuppen1,2, A. Zeddeman2,3, E. K. Kok1, M. van Gent4, K. J. Heuvelman5, M. J. Bart6, H. van der Heide1,7, F. J. van Opzeeland1,7, S. van Selm4,7, M. J. de Jonge1,8, R. de Groot1,8, F. R. Moos1,9, D. A. Davapepaou1,9

1Section Pediatric Infectious Diseases, Laboratory of Medical Immunology, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands; 2Centre for Infectious Diseases Research, Diagnosis, and Screening (IDS), National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands; 3Centre for Infectious Diseases Research, Diagnostics and Screening (IDS), National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands; 4Centre for Infectious Diseases Research, Diagnosis, and Screening (IDS), National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands; 5Centre for Infectious Diseases, Parasitology Unit, Heidelberg University Hospital, Heidelberg, Germany; 6Centre for Infectious Diseases, Parasitology Unit, Heidelberg University Hospital, Heidelberg, Germany; 7German Centre for Infection Research (DZIF), partner site Bonn-Cologne, Germany; 8Centre for Infectious Diseases, Parasitology Unit, Heidelberg University Hospital, Heidelberg, Germany; 9Department of Medicine, University Hospital Muenster, Muenster, Germany

The first B. pertussis vaccines were introduced in the 1940s-1960s and were comprised of killed bacteria (wP vaccines). Despite the high effectiveness of wPs, high reactogenicity led to their replacement with acellular pertussis vaccines (aPs). aPs contain several strains following vaccination with aP but not wP vaccines. Of concern, Prn, a common B. pertussis strain following vaccination with aP but not wP vaccines. Of concern, Prn, a common

WS.D4.01.05

Transphagocytosis, a novel mechanism for bacterial uptake by B cells

R. Garcia Ferreras2, G. Ramírez Santana2, A. Cruz Adalia2, I. Osuna Pérez2, M. Torres Torresano2, R. J. Carrasco2, E. Veiga Chacón2

2Centro Nacional de Biotecnología (CNB), Madrid, Spain; 3ISCIC, Madrid, Spain

It is now well established that B cells are antigen presenting cells (APC). Indeed, it is known that, in vivo, B cells are able to uptake soluble and surface-tethered antigens presented on different types of cells. However, the way B cells capture bacteria remains unknown. Interestingly, we have shown that B cells capture bacteria from previously infected dendritic cells DC). During DC - B cells contact, a process known as transphagocytosis, bacteria were rapidly internalized by B cells. Bacterial antigens resulting from degradation were cross-presented to CD8+ T which resulted strongly activated and proliferated. Taking into account that only very few cells are able to activate naive CD8+ T cells triggering its proliferation, this study demonstrates a potential mechanism to generate a cytotoxic response. Therefore, we characterized that a previous exposition of Pathogen Associated Molecular Patterns (PAMPs) might improve these immune abilities by B cells against infection.
ZIKV infection is a member of the Flaviviridae family and recent outbreaks have shown that it is very infectious and infection has been associated with abnormal fetal brain development. ZIKV is transmitted through mosquito bites and sexual contact. However, little is known about first cell targets and subsequent viral dissemination as well as immune responses induced by infection. Here we investigated the role of dendritic cell (DC) subsets in ZIKV infection of human skin and vaginal mucosa, initial tissues involved in infection. These data suggest human primary DC were isolated from skin, buffy coats and vaginal mucosa and infected with a wild-type ZIKV strain. Moreover, we used an ex vivo infection model from skin and also embryonic neuronal cells as target cells during the different assays. Notably, mucosal and epidermal Langerhans cells did not become infected by ZIKV, whereas in contrast ZIKV efficiently infected human submucosal DCs. Infected DCs transmitted the virus to target cells, suggesting that DCs are involved in ZIKV dissemination. ZIKV infection of DCs induced a strong type I Interferon (IFN) response, DC maturation and expression of pro-inflammatory cytokynes. The type I IFN response limits ZIKV infection as inhibition of type I IFN signaling increases ZIKV survival. These data strongly suggest that mucosal DCs are one of the first target cells for ZIKV and are involved in induction of innate and adaptive immunity to the virus. Dissecting the adaptive immune responses as well as viral dissemination is important to develop strategies to prevent ZIKV-associated pathologies.

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WS.D4.02 Responses to mucosal microbial pathogens

WS.D4.02.01 Combined chemical genetics and data-driven bioinformatics approach identifies receptor tyrosine kinase inhibitors as host-directed drugs for intracellular bacterial infections

C. J. Korbee1, M. T. Heemskerk1, D. Kacew1, E. van Strijen1, D. Rabiez1, K. L. Franken2, L. Wilson3, N. D. Savage1, S. Drorsok1, T. H. Ottenhoff4, M. C. Haks5
1LUMC, Leiden, Netherlands; 2Jozef Stefan Institute, Ljubljana, Slovenia.

Antibiotic-resistance poses rapidly increasing global problems in combating multidrug-resistant (MDR) infectious diseases like MDR tuberculosis, prompting for novel approaches including host-directed therapies (HDT). Intracellular pathogens like Salmonella and Mycobacterium tuberculosis (Mtb) exploit host signaling pathways to survive intracellularly. Thus far, only very few HDT-compounds targeting host pathways have been identified for HDT. In a Library Of Pharmacologically Active Compounds (LOPAC)-based drug-repurposing screen, using a novel intracellular infection screening assay, we identified multiple compounds, which target Receptor Tyrosine Kinases (RTKs) and inhibited intracellular Mtb and Salmonella more potently than currently known HDT-compounds. By developing a data-driven in silico model based on confirmed targets from public databases, we successfully predicted additional efficacious HDT-compounds. These also appeared to target host RTK signaling and inhibited intracellular Mtb including MDR-Mtb. A complementary human kinase siRNA screen independently confirmed the role of RTK signaling and kinases (BLK, ABL1 and NTRK1) in host control of Mtb. These three approaches validate RTK signaling as a new druggable host pathway for HDT against intracellular bacteria (Nature Communications, 2018).

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WS.D4.02 Host-directed, autophagy-modulating compounds restrict intracellular Mycobacterium tuberculosis and Salmonella Typhimurium infection in human macrophages

LUMC, Leiden, Netherlands.

The persistent increase of multidrug-resistant (MDR) Mycobacterium tuberculosis (Mtb) infections negatively impacts tuberculosis (TB) treatment outcomes, prompting for the development of alternative strategies in addition to conventional antibiotics. Host-directed therapies (HDT) pose such an alternative, especially since the success of Mtb can partly be explained by its capacity to alter its host signaling pathways in order to create a pathogen favourable intracellular environment. Similarly, Salmonella infections, causing a.o. typhoid fever, are accompanied by significant mortality and pose therapeutic difficulties because also this pathogen modifies host regulatory networks to ensure its intracellular survival. One key mechanism of host defence is autophagy, which is of particular interest for HDT given its important and versatile functions in TB pathogenesis. Here a commercially available library of compounds with proven and defined autophagy-modulating activity was screened for small molecules capable of inhibiting intracellular (but not extracellular) Mtb and Salmonella Typhimurium (Stn) survival.

Four FDA-approved compounds were found to act against (MDR-)Mtb and Stn in primary human macrophages in a host-directed manner, and displayed synergy with suboptimal doses of Rifampicin. To elucidate their mechanisms of action various functional assays were performed including monitoring of autophagy, lysosomal activity, and reactive oxygen species (ROS) generation. The results showed clear differences in the mechanisms targeted by each of the compounds. Current efforts, including studies in a zebrafish infection model, are directed to further dissect these mechanisms by which these autophagy-modulators decrease intracellular bacterial survival.

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WS.D4.02.03 The Mal-IFN-γ receptor axis receptor axis and TIRAP-S180L polymorphism as determinants of susceptibility to pneumococcal disease

1School of Biochemistry and Immunology, Trinity Biomedical Science Institute - Trinity College Dublin, Dublin, Ireland, 2Institute of Molecular Medicine, Trinity College Dublin & St James’s Hospital – Ireland., Dublin, Ireland, 3Goethe University Frankfurt/Main, Frankfurt, Germany, 4University of Massachusetts Medical School, Division of Infectious Disease & Immunology, Massachusetts, United States.

Genetic single-nucleotide polymorphisms (SNP) affecting immune receptors or their signaling components can influence susceptibility to infections. TIRAP encodes the MyD88-adapter like protein (Mal), a bridging adaptor for TLR4 and TLR2. A SNP in TIRAP resulting in the substitution S180L alters susceptibility to invasive pneumococcal infection, tuberculosis and sepsis. TIRAP10 homozygosity increases susceptibility, while heterozygotes TIRAP6 are protected compared to TIRAP1010 wild-types. We discovered a TLR1-independent role for Mal in tuberculosis whereby it mediates interferon (IFN)-γ receptor (IFNGR) signaling which is affected in TIRAP1010 impairing autophagy and mycobacterial killing in macrophages. Here we investigated the role of Mal and the Mal-IFNγ axis in invasive pneumococci caused by Streptococcus pneumoniae, an important human pathogen that kills >1.6 million people/year.

We showed that Mal is required for protection against pneumococci, as evidenced by the hyper-susceptibility of TIRAP10 mice to invasive pneumococci. Despite the involvement of Mal in TLR2 and TLR4 signalling, Tlr2/4S180L immuno-modulated macrophages (BMMs) expressed comparable Ifna mRNA to wild-types when infected with pneumococci or stimulated with TLR ligands, other than LPS and Pam3CSK. However, TIRAP10 and in particular TIRAP10 over-expressed Ifna and Ifnb mRNA in response to all stimuli, a response that was exacerbated by pre-treatment with IFN-γ. Besides, TIRAP10 and TIRAP10 were less efficient at killing pneumococci in vitro compared to TIR2/4 and wild-types. Finally, infected TIRAP10 mice had increased alveolar infiltration of inflammatory monocytes. These results suggest a novel TLR-independent role for Mal during pneumonia highlighting its key role as a modulator of IFNγ-induced inflammation in the lungs.

WS.D4.02.04 Dual RNA-seq unveils novel host-pathogen interactions during colonic bacterial infections

F. Won; Johns Hopkins University, Baltimore, United States.

Attaching/effacing (A/E) pathogens including enteropathogenic Escherichia coli (EPEC), enterohemorrhagic E. coli (EHEC), and the rodent equivalent Citrobacter rodentium (CR) are important causative agents of foodborne diseases. A/E pathogen infections cause severe morbidity and mortality in immunocompromised hosts with low interleukin-22 (IL-22); however the crucial host-pathogen interactions and the pivotal A/E virulence proteins (effectors) under immunocompromised conditions, remain elusive. We utilize a “dual RNA-sequencing” approach to simultaneously profile gene expression in the pathogen and the host, and identify an extracellular metalloprotease as a novel virulence factor during C. rodentium infection in I22/− mice. Genetic deletion of the extracellular protease substantially attenuates C. rodentium infection-induced morbidity and mortality in I22/− mice, which underscores the pathophysiological relevance of bacterial extracellular proteases. Moreover, the extracellular protease deficiency impedes the induction of pro-inflammatory cytokine gene expression in the C. rodentium-infected I22/− colon. These findings reveal novel host-pathogen interactions during C. rodentium infection in I22/− mice, which could provide novel strategies to control A/E pathogen infections under low IL-22 immunocompromised conditions associated with chronic HIV infection, organ transplantation, and other diseases. Supported in part by NIH Grant GM111682.
The intestinal epithelium constitute a first line of defense against gut microbes, which includes secretion of various antimicrobial substances. Reactive oxygen species (ROS) are well characterized as part of the innate phagocytic immunity; however, a role in controlling microbiota in the gut lumen is less clear. Here, we demonstrate, through in vivo imaging, a remarkably high production of ROS in the ileum of normal healthy mice. The ROS production depends on the enzymes iNOS (NO production) and NOX1 (superoxide production). NO and superoxide rapidly form peroxynitrite. Peroxynitrite is bacterialidal and one of the important ROS in respiratory burst. Mice deficient in iNOS and NOX1 have increased bacterial load and a significant shift in the microbiota composition of the ileum. Furthermore, the ROS appear to prevent reflux of microbiota from large intestine and be of relevance for bacterial overgrowth and translocation. Our data suggests a new role of ROS in regulating the bacterial content at the border of the small and large intestine. This may imply a unique role of ileum in maintaining homeostasis of the gut microbiota through production of ROS to prevent reflux from the large intestine, bacterial over growth and translocation. Furthermore, as several conditions and diseases such as Crohn’s disease and SIBO have been linked to impaired microbial homeostasis in ileum, these findings might provide new insights into pathogenesis related mechanisms that can indicate strategies for disease prevention.

**WS.D4.02.06**

**NLRP11 negatively regulates NF-κB and type I interferon responses**

K. Ellwanger, E. Becker, J. Kienzer, A. Sowa, Y. Postma, Y. Cardona Gloria, A. N. Weber, T. A. Kofer; 1University of Heidelberg, Stuttgart, Germany; 2University of Tübingen, Tübingen, Germany.

Nucleotide-binding domain and leucine-rich repeat containing proteins (NLRs) are a group of intracellular pattern recognition receptors, involved in the regulation and induction of innate and adaptive immunity in mammal. However, only recently a functional characterization of NLRP11, a primate-specific member of the NLR family, was identified. Here, we report that NLRP11 is highly expressed in reproductive tissue and in immune cells, including myeloid cells, B cells and some B cell lymphomas lines. Overexpression of NLRP11 in human B lymphocytes did not trigger key innate immune signalling pathways including NF-κB, type I interferon and caspase-1. By contrast, expression of NLRP11 repressed MyD88-induced NF-κB and TRIF-induced type I interferon responses. This effect was independent of the PYD domain and AT&PAse activity of NLRP11 but mediated by its LRR domain. Accordingly, knock-down of NLRP11 in human myeloid THP1 cells enhanced lipopolysaccharide (LPS) and Sendai Virus (SeV)-induced cytokine and interferon responses upon NLRP11 depletion. In summary, our work identifies a novel role of NLRP11 in the regulation of inflammatory responses in human cells.

**WS.D4.03.01**

**Functional cooperation between complement factor H and the long pentraxin PTX3 in the immune response to Aspergillus fumigatus**

A. Parente, R. Petroni, M. Stravalli, M. Sironi, R. Leone, S. Valentina, A. I. Day, B. Bottazzi, A. Mantovani, A. Ruwwe-Glösenkamp; 1Humanitas Clinical and Research Center, Rozzano, Italy; 2Department of Biomedical Sciences, Humanitas University, Rozzano, Italy; 3Wellcome Trust Centre for Cell-Matrix Research, Division of Cell-Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom.

Aspergillus fumigatus (AF) is the major eukaryotic agent of invasive Aspergillosis (IA), a severe infection amongst immunocompromised individuals. A pivotal role in the host resistance to this pathogen is played by polymorphonuclear neutrophils (PMNs) and complement. The long pentraxin PTX3 exerts opsonic activity towards AF conidia, and enhances their phagocytosis and killing by PMNs via complement pathways. Here we characterized the molecular crosstalk between PTX3 and complement in the opsono-phagocytosis of AF. Complement activation on AF conidia was assessed by Western Blotting and ELISA using complement proteins and human sera depleted of selected complement components in the presence and absence of recombinant PTX3. In parallel experiments, AF phagocytosis and killing by human and murine PMNs was assessed in vivo by Flow Cytometry. We found that PTX3 promotes the selective recruitment of C3b on the conidial wall, by targeting the alternative pathway (AP) of complement. To our surprise, factor H (main inhibitor of AP) was necessary for this activity, thus pointing to a novel function (activating rather than inhibitory) of this factor when combined with PTX3. Consistent with this, factor H and complement receptor 1 (CR1, major receptor of C3b) were required for the pro-phagocytic and pro-killing properties of PTX3. Therefore, a cooperation was observed between factor H and PTX3 with an unexpected functional outcome: enhanced recruitment of C3b on AF. Given the potent opsonic activity of C3b (via CR1), we believe that this is a major mechanism of PTX3 in the promotion of AP phagocytosis and killing by PMNs.

**WS.D4.03.02**

**Orchestration of systemic anti-fungal Th17 immunity and immune response by a single member of the mycobioide**


Introduction: Th17 cells protect against bacteria and fungi, but also contribute to chronic inflammation. In humans, Th17 responses are particularly important against Candida albicans, a common fungal pathobiont. In contrast, the role of Th17 cells for other pathogenic fungal species is less clear. In the lung, fungal-related disorders and sensitizations are often associated with chronic respiratory diseases as asthma, COPD and cystic fibrosis. Also these patients showed increased Th17 cytokine levels, which correlate with disease severity. However, most fungal species used to induce human Th17 responses and their potential contribution to pulmonary diseases is currently unknown.

Methods: We used antigen-reactive T cell enrichment (ARTE) for the ex vivo analysis of human T helper cell responses against 25 common human pathogenic fungal species. Results: We show that C. albicans is the sole direct fungal inducer of Th17 cells in humans. For all other fungi tested, minor and variable fractions of Th17 cells were detected. Surprisingly, these Th17 cells, but not Th1 cells against the same fungal species, were strongly and selectively cross-reactive against C. albicans. Patients with pulmonary inflammation displayed elevated frequencies of cross-reactive A. fumigatus Th17 cells, suggesting their specific contribution to lung pathology. In particular in patients with allergic sensitization to A. fumigatus, increased Th17 responses strongly correlated with acute ABPA. Conclusions: Our data identify C. albicans as the major fungal inducer of human Th17 responses. We provide a unique example how protective Th17 immunity may simultaneously promote immune pathology when deviated to target different antigens and tissues via heterologous immunity.

**WS.D4.03.04**

**Inherited p40phox deficiency differs from chronic granulomatous disease: Distinctive molecular, cellular and clinical phenotypes**

A. van de Geer, A. Nieto-Pattarin, D. Boos, J. Casanova, T. W. Kuipers, J. Bustamante; 1Sanoquin, Amsterdam, Netherlands; 2Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM U1163, Necker Hospital for Sick Children, Paris, France; 3St Gilis Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, United States; 4Emma Children’s Hospital, Amsterdam, Netherlands.

Chronic granulomatous disease (CGD) is a well-defined rare primary immunodeficiency caused by loss-of-function mutations in any of the genes encoding four phagocyte NADPH oxidase subunits (p22phox, p47phox, p67phox, and gp91phox). In contrast, loss-of-function mutations in the NCF4 gene, encoding the p40phox subunit, have only been described once. We report 24 p40phox-deficient patients from 12 additional families in eight countries. These patients display eight different mutations, homozygous in 11 families and compound heterozygous in another. Upon over-expression in NB4 neutrophil-like cells and EBV-transformed B cells in vitro, the mutant alleles were loss-of-function, except for one homoplastic allele. Particle-induced NADPH oxidase activity in the patients’ neutrophils was subnormal, whereas PMA-induced DHR oxidation, a widely used test for CGD diagnosis, was sometimes normal. Moreover, NADPH oxidase activity of EBV-transformed B cells was also subnormal, whereas that of mononuclear phagocytes was normal. Finally, unlike in CGD, neutrophil killing of Candida albicans and Aspergillus fumigatus hyphae was maintained, whereas that of Staphylococcus aureus was impaired both in CGD and in p40phox deficiency. Patients suffer from auto-inflammations and peripheral infections, but not from invasive bacterial and fungal infections. Moreover, all patients are alive at ages 1-46 years and only four of them have received hematopoietic stem cell transplantation. In conclusion, inherited p40phox deficiency underlies a distinctive condition, evoking a mild form of CGD, with inflammatory lesions and peripheral infections, but not with invasive bacterial and fungal disease. Its detection should not rely on the usual clinical test of PMA-induced oxidation of substrates by neutrophils.
WS.D4.03.05
TRIM5α-mediated autophagy: Where HIV-1 restriction and molecular inflammation meet

A. P. Cloherty, J. L. van Hamme, R. Sarrami-Forooshani, N. A. Kootstra, T. B. Geijtenbeek, C. M. S. Ribeiro; University of Amsterdam, Amsterdam, Netherlands.

Contemporary HIV-1 antiretroviral therapy is highly effective, however, treated HIV-1 patients suffer from severe comorbidities due to persistent viral replication and chronic inflammation. There is thus an urgent need to identify therapeutic targets to enhance antiviral immunity and control inflammation in treated HIV-1 patients.

Autophagy functions as an antiviral defense mechanism by degrading viruses and instructing adaptive T-cell responses. We were the first to establish that human TRIM5α mediates assembly of an autophagy activating scaffold to HIV-1 contaminants, which targets HIV-1 for autophagic degradation and restricts infection of a human dendritic cell (DC) subset. Here, we show a novel protective role for autophagy machinery in HIV-1 patients. We have recently identified a gene polymorphism in a regulator of TRIM5α-mediated autophagy that is associated with decreased viral plasma load and improved survival in HIV-1 patients from the Amsterdam Cohort Studies. This polymorphism correlates with increased autophagy levels and heightened CD8+ T-cell responses in vitro, as well as with decreased susceptibility to HIV-1 infection of emigrated DC subsets in ex vivo explant models. These findings underscore the pivotal role for autophagy in limiting HIV-1 replication and boosting antiviral T-cell immunity. Furthermore, our preliminary data identify a novel link between TRIM5α-mediated autophagy and virus-induced molecular inflammation. We have demonstrated that TRIM5α and ATG13 are key in the secretion of inflammatory mediators such as IL-1β and TNFα after viral infection. Hence, our data underscore the in vivo relevance of autophagy mechanisms and the therapeutic potential of targeting autophagy to intervene in acute and chronic HIV-1 infections.

WS.D4.03.06
Type I interferon-dependent NKTosis supports biofilm formation and survival of Pseudomonas aeruginosa during lung infection

E. Plyaeov, S. Bordbar, I. Spyro, S. Long, I. Jablonska; University Hospital, Ear, Nose, and Throat Department, Essen, Germany.

The enhanced predisposition to bacterial complications in cancer or viral infections is known. These clinical situations are often associated with elevated levels of type I interferons (IFN). As neutrophils are the major antibacterial responders in acute phase of infection, we set up to reveal the role of IFNs in the regulation of neutrophil bactericidal properties. In the model of acute Pseudomonas aeruginosa pneumonia we observed elevated bacterial load and lung tissue damage in WT mice, as compared to IFN-deficient animals. We observed enhanced neutrophil extracellular traps (NETs) release by WT lung neutrophils, accompanied by the elevated biofilm formation and survival of P. aeruginosa. Interestingly, infection with biofilm-negative P. aeruginosa revealed no differences in bacterial load between WT and IFN-deficient mice. Treatment of mice with rmIFN-β raised biofilm content and bacterial load in the lung, confirming the role of NKTosis-dependent biofilm formation as supporter of bacterial survival. Summarizing, during Pseudomonas infection IFNs stimulate NETs release by neutrophils, which in turn supports biofilm formation by Pseudomonas. Biofilm protects bacteria from the immune system and leads to their persistence in the lung. Biofilms can also be responsible for the antibiotic-resistance typical for Pseudomonas, therefore targeting NKTosis may help to develop effective treatment strategies for persistent infections with this pathogen.

WS.D4.04 Virus-host interactions

WS.D4.04.01
Human cytomegalovirus reprograms hematopoietic progenitor cells into immunosuppressive monocytes to achieve latency

K. Zen; Nanjing University, Atlanta, China.

The precise cell type hosting latent human cytomegalovirus (HCMV) remains elusive. Here we report that HCMV reprograms human hematopoietic progenitor cells (HPCs) into a unique monocyte subset to achieve latency. Unlike conventional monocytes, this monocyte subset possesses higher levels of IL-17, IL-10 and iNOS, longer lifespan and strong immunosuppressive capacity. CD14+CD33+CD11c+CD73+ peripheral mononuclear cells, is HCMV genome-positive but immediate-early (IE)-negative. Mechanistic studies demonstrate that HCMV promotes the differentiation of HPCs into this monocyte subset by activating cellular signal transducer and activator of transcription 3 (STAT3). In turn, this monocyte subset generates a high level of nitric oxide (NO) to silence HCMV IE transcription and promote viral latency. By contrast, the US28-knockout HCMV mutant, which is incapable of activating STAT3, fails to reprogram the HPCs and achieve latency. Our findings reveal that via activating STAT3/iNOS/NO axis HCMV differentiates human HPCs into a longevous, immunosuppressive monocyte subset for viral latency.

WS.D4.04.02
Killer cell proteases target viral immediate-early proteins to control cytomegalovirus infection in a noncytotoxic manner

L. Shan1, J. Meeldijk2, B. Blijenberg3, A. Hendriks4, J. van den Berg2, A. Surlanska1, T. Stamminger1, M. R. Wills1, N. Bovenschen2; 1University Medical Center Utrecht, Utrecht, Netherlands, 2University of Cambridge, Cambridge, United Kingdom, 3University of Erlangen-Nuremberg, Erlangen, Germany, 4Ulm University Medical Center, Ulm, Germany.

Human cytomegalovirus (HCMV) is the most frequent viral cause of congenital defects and can trigger devastating disease in immune-suppressed patients. Cytotoxic lymphocytes (e.g. CD8+ T cells and NK cells) control HCMV infection by releasing the pore-forming protein perforin and five cytotoxic granzymes (GrA, GrB, GrH, GrK, and GrM) towards virus-infected cells. Perforin facilitates the cellular entry of granzymes, which are believed to kill infected host cells through cleavage of intracellular death substrates. However, it has recently been demonstrated that the in vivo killing capacity of cytotoxic T cells is limited. This raises the question whether cytotoxic lymphocytes can also control HCMV infection in a noncytotoxic manner. In this present study, we demonstrate that (primary) cytotoxic lymphocytes block HCMV dissemination and induce the degradation of viral immediate-early (IE) proteins IE1 and IE2 in HCMV-infected cells in a cell death-independent noncytotoxic manner at low effector:target cell ratios. Interestingly, both IE1 and/or IE2 are directly proteolyzed by all human granzymes, with GrB and GrM being most efficient. GrB and GrM cleave IE1 after Asp184 and Leu185, respectively, resulting in IE1 dislocation, IE1 instability, and functional impairment of IE1 to interfere with the JAK-STAT signaling pathway. Furthermore, GrB and GrM cleave IE2 after Asp173 and Leu174, respectively, resulting in IE2 dislocation and functional abolishment of IE2 to transactivate the HCMV UL112 early promoter. Taken together, our data indicate that cytotoxic lymphocytes can employ noncytotoxic ways to control HCMV infection via granzyme-mediated targeting of indispensable viral proteins during lytic infection.

WS.D4.04.03
Pathological role of anti-CD4 antibodies in HIV-infected immunologic non-responders under viral suppressive antiretroviral therapy

W. Jiang, Z. Luo; Medical University of South Carolina, Charleston, United States.

Abstract

Increased mortality and morbidity occurs in human immunodeficiency virus (HIV)-infected patients who fail to increase CD4+ T cell counts despite achieving viral suppression with antiretroviral therapy [ART]. Here we identified an underlying mechanism. Significantly elevated plasma levels of anti-CD4 IgGs were found in HIV+ immunologic non-responders (CD4+ T cell counts ≤ 350 cells/μl) [median (interquartile range), 91.8 ng/mL (53.2-165.8)] compared to HIV+ immunologic responders (CD4+ T cell counts ≥ 500 cells/μl) [26.0 ng/mL (15.9-81.6)]. Healthy controls and HIV+ non-responders (14.9 ng/mL [9.2-24.1]) (P < 0.001 between non-responders and responders or healthy controls, non-parametric Mann-Whitney test). Higher plasma level of anti-CD4 IgG correlated with blunted CD4+ T cell recovery (r = 0.53, P = 0.0002, Spearman correlation test). Furthermore, purified anti-CD4 IgGs from HIV+ immunologic non-responders induced NK cell-dependent CD4+ T cell cytolyis [19.1% (5.8-30.3) vs. 0.04% (0.03-0.05) for treatment with anti-CD4 IgGs and anti-CD4-depleted IgGs respectively] and apoptosis [36.9% (26.6-44.5) vs. 15.4% (14.3-16.8) for treatment with anti-CD4 IgGs and anti-CD4-depleted IgGs respectively] through antibody-dependent cell-mediated cytotoxicity (ADCC) in vitro. We also found that anti-CD4 IgG-mediated ADCC exerts marginally increased apoptosis on naive relative to memory CD4+ T cells (P = 0.06). Consistently, increased frequencies of CD107a+ NK cells and profound decreases of naive CD4+ T cells were observed in immunologic non-responders compared to responders and healthy controls ex vivo. These data indicate that antireactive anti-CD4 IgGs may play an important role in the blunted CD4+ T cell reconstitution despite effective ART.
WORKSHOPS

WS.D4.04.04

HIV-1 exposure enhances sexual transmission of HCV by inducing Syndecan-4 on Langerhans cells

B. M. Nijmeijer1, R. Sarrami Foroshani2, J. Eder1, G. S. Stobo1, R. R. Schreurs1, S. S. Koekkoek3, R. Molenkamp2, J. Schinkel2, P. Reiss1, M. L. Siegenbeek van Heukelom1, M. van der Valk1, C. M. Reinebo1, T. B. Geijtenbeek4

1Department of Experimental Immunology, Academic Medical Center, Amsterdam Invasion and Immunity Institute, University of Amsterdam, Amsterdam, Netherlands, 2Department of Medical Microbiology, Clinical Virology laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, 3Department of Global Health, Academic Medical Center, Amsterdam Institute for Global Health and Development, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, 4Department of Internal Medicine, Division of Infectious Diseases, Amsterdam Invasion and Immunity Institute, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.

The significant rise in incidence of Hepatitis C virus (HCV) infection among HIV-infected men-who-have-sex-with-men (MSM) suggests that HIV under specific circumstances is transmitted via sexual contact. The mechanisms remain unclear. Here we investigated the molecular mechanism of sexual transmission of HCV. Analyses of mucosal anal biopsies from HIV-1 infected MSM identified Langerhans cells (LCs) within mucosal tissue. However, immature LCs were neither infected nor transmitted HCV to hepatocytes in vitro and ex vivo. As sexual transmission is mostly observed within HIV-1 infected individuals, we pre-exposed tissues with HIV-1 and, strikingly, HIV-1 pre-exposure significantly increased HCV transmission by LCs. HIV-1 replication is crucial for the increased HCV transmission as treating ex vivo tissue with HIV-1 replication-inhibitors significantly decreased HIV-1 induced HCV transmission. Next we identified the mechanism in HIV-1 exposed LCs. Notably, HCV transmission by HIV-1 exposed LCs was dependent on Syndecan-4, as silencing of Syndecan-4 by shRNAs reduced transfer of HCV. Furthermore, we confirmed our findings expressing Syndecan-4. This combination therapy resulted in 88% survival in animals that would otherwise succumb to disease within 36 hours. Treatment effectively reduced the accumulation of cells in inflammatory foci. To this end, we treated mice with neurological signs of CM with 2 doses of IMP in combination with WHO recommended anti-parasitic artesunate. Our data strongly suggest that HIV-1 replication as well as immune activation in mucosal tissues in HIV-1 infected MSM, changes LC function, allowing Syndecan-4 to capture and subsequently transmit HCV to hepatocytes. This novel transmission mechanism implicates also that the activation state of LCs is an important determinant for HCV susceptibility after sexual contact. This work was supported by Aidsfonds, grant number: 2014014

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DUSP4 regulates STING and RIG-I mediated signaling in response to virus infection

H. Jiao1, S. J. James, Y. Zhang

National University of Singapore, Singapore, Singapore.

Detection of cytosolic nucleic acids by pattern recognition receptors including STING and RIG-I leads to activation of multiple signalling pathways that culminate in the production of type I interferons (IFNs) which are vital for host survival during virus infection. In addition, type I IFNs are also associated with autoimmune diseases. Hence, it is essential to elucidate the mechanisms that govern their expression. Using DUSP4 in vitro study, we identified the critical role of DUSP4 in innate immune signalling was demonstrated. It was found that DUSP4 knockout (KO) macrophages expressed increased level of type I IFNs in response to RIG-I and STING activation, as well as influenza and HSV-1 infection compared to wildtype (WT) cells. This increased type I IFN expression in KO cells was associated increased activation of ERK and TBK1-IRF3 in KO cells compared to WT cells. Mice deficient in DUSP4 are more resistant to both RNA and DNA virus infection but are more susceptible to malaria compared to control. Mechanistically, DUSP4 inhibits TBK1 and ERK2 activation to suppress type I IFN production. Therefore, our study not only established DUSP4 as a common regulator of nucleic acid sensor signalling, but also shed light on the regulation of the type I IFN system.

WS.D4.04.06

A IncRNA promotes viral replication by modulating host cellular metabolism

P. Wang1, J. Xu1, W. Wang2, X. Cao2,3

1Institute of Immunology, Second Military Medical University, Shanghai, China, 2Institute of Immunology, Zhejiang University School of Medicine, Hangzhou, China, 3Department of Immunology & Center for Immunotherapy, Institute of Basic Medical Sciences, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China.

Viruses have evolved to alter the host metabolic pathways to ensure optimal niche for viral replication and survival. Identification of how viruses alter the host cellular metabolism is crucial to help us understand the pathogenesis of viral infection and revealing targets for antiviral therapeutics. We previously demonstrated that long non-coding RNAs (lncRNAs) regulate dendritic cell differentiation, and therefore, have a pleiotropic effect to the host immune system (1). Here we hypothesized that viruses utilize IncRNA to alter the host cellular metabolism that provides necessary energy and material for viral replication. In this study, we identified that a group of lncRNAs were induced by the infections from a number of viruses. Functional screening demonstrated that the expression of one of these viral-induced lncRNAs, IncRNA-ACOD1, enhanced viral replication in both murine and human cells. IncRNA-ACOD1 knockout mice, viral infection was significantly reduced in an IFN-independent manner. Gene expression profiling and metabolomics surveys revealed that viral infections led to significant host metabolic changes in wildtype hosts but the IncRNA-ACOD1 knockout totally abolished the metabolic alteration. Mechanistically, we demonstrated that IncRNA-ACOD1 directly binds to a metabolic enzyme, G6PD2. The binding region on G6PD2 is spatially adjacent to its substrate-binding site, and thus, the binding of IncRNA-ACOD1 enhanced G6PD2’s catalytic activity. The treatment of G6PD2 or its metabolites restored viral replication in IncRNA-ACOD1 knockout mice, demonstrating that IncRNA-ACOD1 mediated viral replication is dependent on G6PD2 catalysis. This work revealed a novel feedback mechanism of viral infection by altering host cellular metabolism through an IncRNA.


WS.D4.05.05

Innate-adaptive interface during microbial infections

WS.D4.05.01

Targeting newly identified pathogenic monocyte subsets with immune-modulatory treatment resolves severe malaria

P. Niewold1, A. Cohen2, C. van Vreden1, D. R. Getts1, G. Grau1, N. King1

1University of Sydney, Sydney, Australia, 2Ramacotti Facility for Human System Biology, Sydney, Australia, 3Northwestern University, Chicago, United States.

Severe malaria is caused by the mosquito-borne parasite Plasmodium falciparum and is associated with lethal complications including acute respiratory distress syndrome and cerebral malaria (CM). Current treatment has limited efficacy in advanced infection as it is primarily directed at the parasite. This fails to address the neurological damage and respiratory distress caused by host blood cell accumulation in the brain microvasculature and lung interstitium. Therefore, we sought to identify cells contributing to pathology as novel targets for immunomodulatory therapies. In a preclinical mouse model of CM, high-dimensional flow cytometry and computational analysis identified Ly6C+ monocytes and interstitial macrophages as the main pathological populations in the brain and lungs, respectively. Previous studies have shown immune modifying particles (IMP) target Ly6C+ monocytes, preventing their migration to inflammatory foci. To this end, we treated mice with neurological signs of CM with 2 doses of IMP in combination with WHRecommended anti-parasitic artemesine. This combination therapy resulted in 88% survival in animals that would otherwise succumb to disease within 36 hours. Treatment effectively reduced the accumulation of cells in the brain vasculature and lung, resulting in clearance of parasitemia and immunity.

In conclusion, through detailed analysis of the immune response in severe malaria, we identified principal pathogenic immune subsets and targeted them with a novel combination therapy, resulting in increased survival in an otherwise lethal syndrome. This is the first specific immunemodulatory treatment addressing both hostmediated pathology and the parasite in severe malaria, revealing a novel avenue for human treatment.

WS.D4.05.02

The use of mass cytometry to study type 2 immune responses in the context of helminth infections

K. de Ruiter1, D. L. Tahapory1, K. Starn1, V. van Oenen1, E. Sartono1, J. W. Smits1, T. Supali1, M. Yazdanabaksh4

1Leiden University Medical Center, Leiden, Netherlands, 2Universitas Indonesia, Jakarta, Indonesia, 3Radboud University Medical Center, Nijmegen, Netherlands.

Studies have shown that helminths can have a profound role in shaping immune responses to vaccines, allergens or autoantigens by inducing strong type 2 and regulatory responses. We used mass cytometry to gain insight into the immunomodulatory effects of helminths, by performing unbiased immune profiling of Indonesian adults who were infected with soil-transmitted helminths, before and 1 year after anthelmintic treatment. We then used high-dimensional single-cell immunological data, allowed the identification of very distinct immune signatures in Europeans and Indonesians. Higher frequencies of Th2 cells, in particular CD161+ Th2 cells, LC2s and regulatory T cells expressing CTLA-4 and ICOS were found in Indonesians that were infected, compared to Europeans. Except for IC2s, the frequencies of these cell subsets decreased after 1 year of anthelmintic treatment. In addition, we assessed the functional capacity of cells, and observed a clearly higher proportion of Th2 and regulatory T cells upon stimulation with PMA and ionomycin in infections further decreased after deworming. Conversely, CD4+ and CD8+ T cells, γδ T cells and ILC2s showed to be the main producers of Th2 cytokines. These results provide us with a detailed insight into the types of cells that participate in the strong type 2 and regulatory response induced by helminths.

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106

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Type 2 inflammation is characterized by production of the cytokines IL-4, IL-5 and IL-13 and promotes clearance of gastrointestinal helminth parasites, which infect over 2 billion people worldwide. Basophils are innate immune cells that drive expulsion of the murine helminth *Trichuris muris*. However, the molecular mechanisms that control basophil function and gene expression during helminth-induced type 2 inflammation remain unclear. We show that during *T. muris* infection, basophils localized to the intestine and underwent profound remodeling of the Notch signaling pathway, which regulates gene expression programs during development and inflammation. In vitro, Notch inhibition abrogated IL-33 elicited basophil IL-4 and IL-6 production by directly targeting If4 and If6. Transcriptional profiling of Notch-deficient basophils revealed that Notch directs basophil responsiveness to inflammatory cues and effector gene expression. In vivo, Notch-deficient basophils did not localize effectively within the lamina propria of the intestine, at the site of infection, and displayed decreased interaction with CD4+ cells. Consequently, mice lacking basophil-intrinsic Notch signaling had impaired worm clearance and decreased induced type 2 inflammation following *T. muris* infection. These findings demonstrate that Notch regulates basophil gene expression and effector function during helminth-induced type 2 inflammation, with repercussions for our understanding of type 2 immunity and for development of effective therapeutics aimed at this arm of host defense.

Type 1 Interferons Suppress Anti-parasitic CD4+ T Cell Responses in Visceral Leishmaniasis

R. Kumar1, P. Buret2, F. Rivera3, N. Singh4, S. Sundar1, C. Engwerda5;
1Institute of Science, Banaras Hindu University, Varanasi, India; 2QIMR Berghofer Medical Research Institute, Brisbane, Australia; 3Institute of Medical Science, Banaras Hindu University, Varanasi, India.

Introduction: Many pathogens, including viruses, bacteria, and protozoan parasites, suppress cell mediated immune responses through activation of type I Interferon (IFN-1) signalling. However, the role of IFN-1 during *Leishmania donovani* infection causing visceral leishmaniasis (VL) is not well known. Here we report that IFN-1 plays an important role in the pathogenesis of VL by impairing parasite clearance and suppressing pro-inflammatory cytokine production. Methods: Mice lacking type-1 IFN signalling (B6.129Nrf1−/− mice) and wild type (WT) C57BL6/J mice were infected intra-venously with 2×10^7 *L. donovani* amastigotes. Parasite burden was measured at day 14, 28 and 56 post infections and serum cytokine levels was analysed. Intracellular cytokine staining and flow cytometry was performed to detect the CD4+ T cell-derived IFN-γ production. Peripheral blood mononuclear cells (PBMCs) from VL controls were also collected to measure mRNA encoding IFN-1 related genes and whole blood assay was employed to measure the antigen specific immune response after IFN-1 signalling blockade. Results: B6.129Nrf1−/− mice showed enhanced pro-inflammatory cytokine production and better control of parasite burden in liver and spleen. IFN-1 signalling suppressed CD4+ T cell-derived IFN-γ production and prevented Th1 response from controlling parasite replication. Studies in VL patients supported these findings and showed enhanced accumulation of mRNA encoding type-1 IFN signature genes in PBMCs that were reduced following successful drug therapy. The blockade of type-1 IFN signalling enhanced antigen specific IFN-γ production. Conclusion: Together, these results identify type-1 IFN signalling pathways as a potential therapeutic target to treat VL by enhancing anti-parasitic CD4+ T cell responses.

Memory CDB T cell infection versus tissue resident memory T cells: same patroliers, same controllers?

S. P. M. Welten, A. Oenens;
Institute of Microbiology, ETH Zürich, Zürich, Switzerland.

The induction of memory CDB T cells residing in peripheral tissues is of considerable interest for T cell based vaccines as they can immediately exert effector functions and thus provide protection in case of pathogen encounter at mucosal and barrier sites. Cytomegalovirus (CMV)-based vaccines support the induction and accumulation of large amounts of effector memory CDB T cells in peripheral tissues, a process called memory inflation. Tissue resident memory (T RM) T cells are another subset of cells that take long-term residence in peripheral tissues. Both populations have gained substantial interest in exploiting for vaccine purposes; however, it is unclear which population is superior in providing long-term effector memory CD8 T cell responses in the lungs gradually declined over time. The different characteristics of T RM and inflammatory T cells also translated in distinct capacities to control various viral infections. Together these results bear relevance for vaccines aimed at eliciting memory T cells at mucosal sites.

Bacterial Infections and immune activation

A novel mechanism of neutrophil entry into epithelial cells is involved in damping inflammation but exposes a backdoor for bacterial invasion

N. Y. SHPigel
Hebrew University, Rehovot, Israel.

Neutrophil mobilization is a crucial response to protect the host against invading microorganisms. Neutrophil recruitment and removal is tightly regulated through apoptosis and phagocytosis by resident and recruited macrophages. Nevertheless, in many organs, macrophage mobilization across the barrier epithelium is dearth and cannot be a major mechanism for homeostasis of inflammation. We suggest that in such mucosal surfaces or barrier epithelium, like urinary and gall bladder or the mammary alveoli, phagocytosis of apoptotic neutrophils by macrophage may not be the only or most important mechanism of neutrophil safe disposal and homeostasis of inflammation. Here we propose a novel and previously unrecognized mechanism of neutrophil internalization and phagocytosis by epithelial cells. Viable neutrophils trigger a mechanism that enables them to crawl into cytoplasmic double membrane compartments in the host cells. Next, internalized neutrophils lose the membrane compartment and undergo apoptotic cell death in the cytoplasm. We were able to demonstrate this incredible phenomena both in vitro on epithelial cell lines and in murine in vivo systems. This mechanism contributes to safe disposal of neutrophils and for the resolution of inflammation in some organs and disease processes. Moreover, some pathogenic bacteria take advantage of neutrophil cell invasion properties to invade and proliferate in the epithelial cells, invasion to epithelium may be of prime importance in the pathogenesis of major diseases such as bovine mastitis, urinary tract infection and typhoid fever, and might account for the chronic carriage and relapsing disease.
Lung surfactant lipids provide immune protection against non-typeable Haemophilus influenzae respiratory infection


1CIBER de Enfermedades Respiratorias, Madrid, Spain, 2Universidad Complutense de Madrid, Madrid, Spain, 3The Francis Crick Institute, London, United Kingdom, 4Instituto de Agrobiotecnología, CSIC-Universidad Pública de Navarra, Mutixa, Spain.

Non-typeable Haemophilus influenzae (NTHi) causes persistent respiratory infections in immunocompromised patients likely linked to its capacity to invade and survive within pneumocytes. In the alveolar fluid, NTHi is in contact with pulmonary surfactant, a lipoprotein complex that prevents alveolar collapse and constitutes the front line of defense against inhaled pathogens. The objective of this study was to investigate the effect of surfactant phospholipids on the host-pathogen interaction between NTHi and pneumocytes. For this purpose, we used two types of surfactant lipid vesicles present in the alveolar fluid: i) multilamellar vesicles (MLVs, >1 μm diameter), which constitute the tensiveoactive material of surfactant, and ii) small unilamellar vesicles (SUrs, 40 nm diameter), which are generated after inspiration/expiration cycles, and are endocytosed by pneumocytes for their degradation and/or recycling. Results indicated that MLVs of surfactant inhibited adhesion of NTHi to pneumocytes and, consequently, NTHi invasion. In contrast, endocytosed surfactant lipids did not affect NTHi adhesion but inhibited entry of NTHi pneumocytes. SUVs of lung surfactant were endocytosed via the scavenger receptor SR-BI, but not CD36, and anti-SR-BI antibodies abrogated surfactant inhibition of NTHi invasion. Endocytosis of SUVs inhibited Akt phosphorylation and Rac1 GTPase activation, key events in NTHi internalization. Lung surfactant administration in a mouse model of NTHi pulmonary infection amplified bacterial clearance, supporting the protective role of surfactant lipids against NTHi infection. These results suggest that decreased surfactant lipid levels reported in smokers and patients with chronic obstructive pulmonary disease may increase their susceptibility to infection by NTHi. Funding: SAF-2015-65307-R.
WORKSHOPS

WS.D4.05.06
Analysis of C6G-based peptides in experimental sepsis
C. Cataldi1, M. Martínez-Flores2a, M. Velasco-de Andrés1, O. Cañadas1b, V. Fraile-Ágreda1b, S. Casado-Lombart1, N. Armiger-Barrós1, M. Consuegra-Fernández1, C. Casalí1c, F. LOZANO1d,e,2
1Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, 2Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain, 3Departamento de Bioquímica y Biología Molecular, Universidad Complutense de Madrid, Madrid, Spain, 4Servei d’Immunologia, Centre de Diagnòstic Biomèdic, Hospital Clinic de Barcelona, Barcelona, Spain, 5Departament de Biomedicina, Hospital del Mar, Universitat de Barcelona, Barcelona, Spain.

Sepsis still constitutes one of the most important causes of death worldwide, a fact aggravated by the appearance of multidrug-resistant strains due to indiscriminate use of antibiotics. Receptors from the innate immune system recognize pathogen-associated molecular patterns (PAMPs) and can be a source of antimicrobial therapies alternative/additive to antibiotics. C6G is a lymphocyte-specific surface receptor of the scavenger receptor cysteine-rich superfamily (SRCR-SF) displaying bacterial-binding properties through recognition of PAMPs from Gram positive (lipoteichoic acid; LTA) and negative (lipopolysaccharide; LPS). The present work analysed the bacterial-binding properties of three conserved short peptides (11-mer) mapping at surface accessible locations from each of the three extracellular SRCR domains of human C6G (CD6.PD1, GTVEVRLEASW; CD6.PD2 GRVEMLEHGEW; and CD6.PD3, GQVEVHFRGVW). All peptides showed relative high binding affinities for both LPS (Kd from 3.5 to 3.000 nM) and LTA (Kd from 36 to 680 nM). CD6.PD1 and CD6.PD3 peptides, but not CD6.PD2, also showed broad in vitro bacterial-agglutination properties.

In vitro studies, the CD6.PD3 peptide excelled by dose- and time-dependent improvement of the survival of mice undergoing cecal ligation and puncture (CLP)-induced sepsis, a fact concomitant with decreased pro-inflammatory cytokine serum levels and bacterial load. CD6.PD3 also showed additive effects on survival of CLP-septic mice when combined with the broad spectrum bactericidal antibiotic Imipenem/Cilastatin. These results illustrate the therapeutic potential of peptides retaining the bacterial-binding properties of native C6G. Supported by Spanish MINECO (SAF2016-80535-R; PCIN-2015-070) and ISCIII [RD12/0015/0018 and CIBERES CB06/06/0002] -co-financed by European Development Regional Fund “A way to achieve Europe”.

WS.D4.07.01
Primary neutrophil defects reveal the mechanisms of neutrophil extracellular trap (NET) formation and NETois: a unique role for active cytoskeletal rearrangements
E. Sprenkeler1, A. Toof1, T. K. van den Berg1, T. Kuipers1,2
1Department of Blood Cell Research, Sanquin Research, and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, 2Emma Children’s Hospital, Amsterdam University Medical Center (AUMC), University of Amsterdam, Amsterdam, Netherlands.

Although the formation and microscopic appearance of neutrophil extracellular traps (NETs), the physiological mechanisms triggering NET release and the concomitant cell death known as NETois, have been studied since has been studied since the first report in 2004. The relevance of NET formation to disease and clinical raritability of this unconventional cellular mechanism remain poorly understood. Using neutrophils of patients with various rare genetically well-characterized disorders as human knockout models (including Chronic Granulomatous disease [CGD], granule deficiencies [GPS], degranulation defects [FHL5], ARPC1B and MKL1 deficiency), we have been able to unravel the consecutive steps required for NETois to occur. Additional use of small compounds to inhibit specific cellular signaling pathways in neutrophils from healthy controls were used to verify the role of these mechanisms identified in patient neutrophils. Time courses indicated differences in the moment certain processes became active from cellular activation until DNA release using microscopy, Sytox Green kinetic determination and live cell imaging during the process of NETois. In this way, we have been able to dissect two phases and the signaling involved in the overall process of NETois. In either of these two phases, our studies demonstrate that results from both species, serine proteases as well as specific granule components and the rearrangements of cytoskeletal elements have an essential and unique contribution in the active DNA release from human neutrophils. These data will be discussed in the view of the clinical manifestations.

WS.D4.07.02
Characterization of neutrophils generated in vitro from Hoxb8-transduced myeloid progenitor cells
A. Orosz, A. Mócsai
Semmelweis University, Budapest, Hungary.

Background: Acute inflammation and neutrophil granulocytes have long been mentioned together, however the molecular background of the neutrophilis’ function is mostly unknown. Therefore we are hoping to uncover these molecular mechanisms using ex vivo generated neutrophil cells from the so called SFC ER-Hoxb8 progenitors. These bone marrow-derived progenitors are retrovirally transduced to express the Hoxb8 transcription factor in the presence of β-estrone, in order to keep them in progenitor state for long periods of time. From them, unlimited amounts of neutrophils can be differentiated using certain cytokines. Materials and methods: Neutrophils were generated via growing progenitors in β-estrone free medium supplemented with G-CSF. Cell surface markers were detected using flow cytometry. ROS production was measured according to cytochrome-c reduction. Adhesion and migration capabilities were tested using different stimulating agents. Fagocyting properties were measured using fluorescent Phalloidin staining. Results: CD11b+ progenitor cell line can be cultured for long time. Ly6G+ neutrophils start to differentiate in 4 days. 5-6 days old neutrophils show strong adhesion upon PMA and IC stimulation. Hoxb8 neutrophils can also produce ROS upon various stimuli in vitro. Neutrophil appear in the circulation after adoptive transfer of progenitors into lethally irradiated recipient mice. In vivo generated neutrophils can migrate and carry out phagocytosis in inflammatory conditions. Conclusions: The SFC ER-Hoxb8 cell line can be a great alternative to the commonly genetically modified mouse-based models, in use to discover the molecular basis of the role of the neutrophils in inflammatory diseases both in vitro and in vivo.

WS.D4.07.03
Regnase-3 is an essential RNase for the Interferon pathway in tissue macrophages
M. Von Gammon1, A. Schaub1, L. Lichti1, M. Tschoj2, V. Hornung1, C. Schülke1, M. Heikenwalder1, E. Glasmacher1
1Helmholtz Center Munich, Munich, Germany, 2Gene Center, Munich, Germany, 3LMU, Munich, Germany, 4DKFZ, Heidelberg, Germany.

Regnases are immune cell-expressed RNases. Regnase-1 degrades cellular and viral RNAs in macrophages and T cells, but the role of Regnase-3 remains unknown. Regnase-3-deficient (Reg3−/−) mice develop severe lymphadenopathy, caused by extramedullary hematopoiesis and increased type-I interferon signaling. CD19, as well as CD4+ specific ablation of Regnase-3 in mice does not result in phenotypic changes, however Ly6M-specific deletion recapitulates this phenotype. Regnase-3 protein is regulated via IKK upon TLR activation, similarly to Regnase-1. However, Regnase-3 steady-state expression is specifically high in macrophages and in non-lymphoid tissues, different from Regnase-1. Whereas Regnase-1 transcription is controlled via NF-kB activity, Regnase-3 is transcriptionally regulated via TLR3 and TBK signaling. Although Regnase-3 deficiency does not affect phagocytic activity, it localizes to phagosomes in macrophages, regulated via lysosomal degradation upon RNA agonist stimulation and interacts with TLR3. Therefore, Regnase-3 is the evolutionary counterpart to Regnase-1, functioning in the endocytosis and interferon pathway, ensuring tissue homeostasis.

WS.D4.07.04
A viral immunoevasin controls innate immunity by targeting the prototypical natural killer cell receptor family
1University of Toronto, Toronto, Canada, 2Manosh University, Clayton, Australia, 3University of Ottawa, Ottawa, Canada, 4University of Rijeka, Rijeka, Croatia.

Natural killer (NK) cells play a key role in innate immunity by detecting alterations in self and non-self ligands via paired NK cell receptors (NKR). Despite identification of numerous NKR-ligand interactions, physiological ligands for the prototypical NK1.1 orphan receptor remain elusive. Here, we identify a viral ligand for the inhibitory and activating NKR-P1 (PD1) in the gastrointestinal cytomegalovirus (CMV) capsid protein m12, a viral NK cell effector function by directly engaging the inhibitory NKR-P1B receptor. However, m12 also interacts with the activating NKR-P1A/C receptors to counter-balance M12 decoy function. Structural analyses reveal that m12 sequesters a large NKR-P1 surface area via a “polar claw” mechanism. Polymorphisms in, and the viral m12 protein and host NKR-P1B/C alleles impact NK cell responses in vivo. Thus, we identify the long-sought foreign ligand for this key immunoregulatory NKR family and reveal how it controls the evolutionary balance of immune recognition during host-pathogen interoper.
Intracellular lifestyle of the probiotic bacteria Lactobacillus plantarum: implications for its extraintestinal dissemination


1Macrophage and T Cell Vaccine Laboratory, CIC bioGUNE, Derio, Spain, 2Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain, 3CIC bioGUNE, Derio, Spain, 4Departamento de Tecnologia de Alimentos, Instituto de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain, 5Department of Nutrition, Food Science and Food Technology, Complutense University of Madrid, Madrid, Spain, 6Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, United States, 7kerbasque, Basque Foundation for Science, Bilbao, Spain.

The interaction of the immune system and microbiota is paramount for the development of human homeostasis. In recent years bacterial symbionts have been found in previously considered sterile body sites, including human breastmilk. Therefore, it seems clear that translocation of bacteria occurs physiologically and this has been hypothesized to be controlled by immune cells. Here, we explore the ability of the probiotic bacterium Lactobacillus plantarum to survive within macrophages and provide some of the mechanisms involved in this process.

We show the ability of L. plantarum strains to survive within the RAW264.7 macrophage-like cell line up to 24 h and, to a lesser extent, inside mouse bone marrow-derived macrophages and human monocyte-derived macrophages. In the absence of antibiotics, L. plantarum strains are capable of extruding from immune cells after internalization, which is consistent with the extraintestinal translocation hypothesis. In the absence of antibiotics, L. plantarum internalization and survival: we found that both processes are dependent on bacterial opsonization and recognition by macrophage complement receptor 3. Additionally, by using human whole blood as a more complex model we observed that monocytes are capable of internalizing and sustaining a significant L. plantarum population.

Collectively, we provide one of the first evidences of the capacity of probiotic bacteria to survive within macrophages, which we proved to be complement-dependent. Therefore, we propose that monocytes/macrophages are important players during physiological bacterial translocation.

WS.D4.07.06
Nanovaccines to prevent listeriosis in the elderly

C. Alvarez-Dominguez, R. Calderon-Gonzalez, H. Teran-Navaora, D. Salcines-Cuevas, M. Fresno-Escudero

1Instituto de Formacion e Investigacion Marques de Valdecilla, Santander, Spain, 2Centro de Biologia Molecular Severo Ochoa, Madrid, Spain.

Introduction: Clinical cases of listeriosis in the elderly are associated with meningitis or septicaemia. Listeriosis severe cases have increased in European countries, Spain in particular urging for safe vaccines. We have prepared new nanovaccine formulations for Listeria monocytogenes (LM) including two different adjuvants. Methods: We have i.v vaccinated old-mice, young adults and pregnant mothers and challenged them with a sub-lethal dose of wild type LM. Brains, livers in neonates and livers and spleens in adult mice are extracted after 6h post mortem, immune responses to LM were evaluated, and immune responses in sera and monocyte derived dendritic cells (MoDC). Results: Old-mice and young adults become protected with nanovaccine formulations using adjuvants that expands CD4 and CD8 immune responses, whereas neonatal mice were not affected by adjuvants that only induce CD8 immune responses. Clinical cases of listeriosis in 2013-2015 and mice sera have defined two useful prognostic immune biomarkers to design listeriosis vaccines: high anti-GAPDH antibody titers and tumor necrosis factor (TNF)/interleukin (IL) 6 ratios. Discussion: Gold glyco-nanoparticles vaccines conjugated to short LM peptides and formulated with a pro-inflammatory Toll-like receptor 4 targeted adjuvant protects safely against elderly and neonatal listeriosis. Mice vaccinated with these nanovaccines did not develop listeriosis or brain diseases and immune responses shifted towards Th1/IL-12 pro-inflammatory cytokine profiles and high production of anti-LM antibodies, suggesting good induction of LM-specific memory. Moreover, these nanovaccine formulations were able to activate Th1 production of monocyte dendritic cells from listeriosis patients, suggesting they might be a good nanovaccine formulation to implement LM-specific immunity in the elderly.

WS.E1.01 Visualizing immune responses

WS.E1.01.01
A hot trick for efficient peptide exchange on MHC class I multimers

J. J. Luimstra, M. A. Grasstal, M. C. Roeze, F. H. Folkenburg, J. Neefjes, H. Ovaa

1Leiden University Medical Center, Leiden, Netherlands, 2X’an jiaostong University, Xi’an, China.

Fluorescently-labelled major histocompatibility complex class I (MHC I) multimers are widely used for the detection, isolation and analysis of T cells in infection, autoimmunity and cancer. We developed temperature-induced peptide exchange as a fast and flexible approach to generate large sets of MHC I multimers with different specificities in parallel. We designed conditional ligands for two dominant alleles, H-2K and HLA-A*02:01, that form stable complexes at 4°C, but dissociate from MHC I at a defined elevated temperature to be exchanged for a peptide of choice. We quantified the peptide exchange by HPLC and mass spectrometry and found that upon temperature exposure, the conditional ligand could efficiently be exchanged for both high- and low-affinity peptides. We performed peptide exchange on prefolded MHC multimers and used them directly to detect CD8+ T cells responses to viral epitopes in mice infected with lymphocytic choriomeningitis virus or cytomegalovirus. We next put our multimers to clinical practice by monitoring the efficacy of adoptive T cell transfer in control of cytomegalovirus reactivation in an allogeneic stem cell transplant recipient. These data illustrate the flexibility and simplicity of using our temperature-exchangeable MHC I multimers. With this strongly improved multimer technology, high-throughput assessment of T cell responses in small sample sizes with become feasible. We will provide examples of this.

WS.E1.01.02
Chemokines and integrins independently tune actin flow and substrate friction during intranodal migration of T cells


1Institute of Science and Technology Austria, Klosterneuburg, Austria, 2Theodor Kocher Institute, University of Bern, Bern, Switzerland, 3Institute of Scientific Instruments of the Czech Academy of Sciences, Brno, Czech Republic

Although much is known about the physiological framework of T cell motility and numerous rate-limiting molecules have been identified in loss-of-function approaches, an integrating functional concept of T cell motility is lacking. Here we used intranodal migration of T cells as a model system to investigate the role of chemokines and integrins in regulating T cell motility. We show that while chemokines independently tune the actin flow, integrins mediate substrate adhesion. Therefore, we propose a new model for T cell motility, where both chemokines and integrins independently tune actin flow and substrate friction during intranodal migration of T cells.

WS.E1.01.03
Inside the infected lung - Watching immune cells battle Aspergillus fumigatus

S. Henneberg, S. Kragmann, L. Bornemann, M. Hasenberg, J. Weish, A. Hasenberg, M. Gunzer

1Institute for Experimental Immunology and Imaging, University of Duisburg Essen, University Hospital Essen, Essen, Germany, 2Microbiology Institute – Clinical Microbiology, Immunology and Hygiene, University Hospital and Friedrich-Alexander-University of Erlangen-Nürnberg, Erlangen, Germany.

Infection with the ubiquitous mold Aspergillus fumigatus is a life-threatening disease for immunosuppressed patients. Inhaled conidia can germinate in the lung and invade the tissue, which causes the invasive aspergillosis (IA). Because its diagnosis is time consuming and unreliable, IA is often diagnosed too late and the mortality rate reaches up to 95%. For the prevention of IA, a better understanding of the normally effective pulmonary immune response is essential. Since immune responses in the lung are regulated tightly by the microenvironment, the effect of an infection on immune cell functionality is best analysed in vivo.

Therefore, we established a novel intravital lung imaging technique that does not irritate the lung and maintains physiological conditions during image acquisition by avoiding a pneumothorax. By triggering recordings at defined and short-time stable lung dilations prior to exhalation via a feedback of the ventilated lung, we advanced microscopy setup allows focus-stable documentation of immune cell behaviour during an infection. With a blood vessel staining and the usage of mouse lines with conditional expression of fluorescent proteins, we are able to study neutrophils and macrophages inside the lung tissue. First promising results exhibit changes of the recruitment, invasion, interaction and uptake of inhaled conidia of these immune cells. Our novel approach allows the visualisation of pulmonary immune cell function at almost physiological conditions in vivo.

This will open up new possibilities for the research and the understanding of pulmonary immunity and ultimately also the prevention and protection of dangerous infections.

110

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

WORKSHOPS
Imaging of cytotoxic antiviral immunity while considering the 3R principle of animal research

F. Luciani1,2, A. Eltahla1, A. McFarlane2, T. van den Hoorn2, J. Neefjes2,3,4, A. R. Lloyd1, D. Busch1,2, K. Shober1, M. Neilson1,2, M. Effemberger1, A. R. Lloyd1, D. Busch1,2, M. Effemberger1, A. R. Lloyd1, D. Busch1,2

1School of Medical Sciences, Sydney, Australia; 2Technical University Munich, Munich, Germany; 3Technical University Munich, Munich, Germany; 4Imperial College, London, United Kingdom.

Cytotoxic CD8 T cells play a key role in controlling viral infection by killing infected cells and establishing protective immunological memory. The affinity and diversity of T cell receptor (TCR) are critical factors that drive the activation and differentiation of CTL, but the molecular mechanisms underlying these dynamics remains elusive. We developed a novel single cell approach to measure and link TCR affinity, gene profiles using single-cell (sc)RNAseq, and functional status of CTL. We analysed >1000 antigen specific (Ag) CD8 T cells using autologous tetramers derived from longitudinal samples of early-phase HCV infections. By using VDJpuzzle, a bioinformatics pipeline to reconstruct full-length TCR from scRNAseq we surprisingly discovered in one subject a monoclonal CD8 T-cell response targeting an HLA-I-B07 restricted peptide (GPR) with high IFN-γ (1600 SFU/10^6 cells) within alveolar capillaries, where neutrophils are able to move quickly. Furthermore, we found that nearly all NK cells in the lung are intravascular. NK cells and neutrophils frequently interact at 5-10 minutes in capillaries and occasionally material is transferred from neutrophils to NK cells. NK depletion resulted in marked changes to neutrophil dynamics such as track length and duration (48.7±16 to 112.2±15μm and 2.50±0.3 to 4.77±0.7min respectively). Stimulation with endotoxin in NK depleted mice lead to a more pronounced increase in neutrophil numbers compared to control mice. We will present data on new routes and pathways in control of MHC class II antigen presentation. This will yield new leads for manipulating the immune system in the case of autoimmune diseases, infections and tumor immunology.
WORKSHOPS

WS.E2E3.01.03

Adaptive Single-Waved Multivariate Algorithm for Mining and profiling High-dimensional Flow Cytometry Data

1Radboud university medical center, Department of Laboratory Medical Immunology, Nijmegen, Netherlands;
2Radboud university medical center, Centre for Molecular and Biomolecular Informatics, Nijmegen, Netherlands;
3University Hospital Essen, Department of Dermatology, Essen, Germany;
4Radboud university medical center, Department of Dermatology, Nijmegen, Netherlands;
5Radboud university medical center, Department of Gastroenterology and Hepatology, Nijmegen, Netherlands.

Introduction: Flow cytometry is an important technology for the diagnosis of life threatening diseases and has great potential for immune profiling of immune mediated inflammatory diseases (IMIDs). The current definition of diseases is largely based on 14th century medicine that is mostly based on the clinical manifestations but not on complex cellular and molecular interactions underlying disease pathology. Flow cytometry might provide IMID associated immune profiles that support diseases stratification, efficient therapy selection and therapy monitoring. Here we used flow cytometry combined with a semi-supervised multivariate algorithm and a machine learning tool to generate immune profiles in order to classify a variety of IMIDs.

Materials: Whole blood and peripheral blood mononuclear cells (PBMCs) from healthy controls, psoriatic (Ps), atopic dermatitis (AD) and inflammatory bowel disease (IBD) patients were collected and stained with five 10-color flow cytometry panels (1.General adaptive/innate cells, 2. T-cell differentiation/maturaiton, 3. B-cell differentiation/maturaiton, 4.T-helper cells and 5.Regulatory T-cells), measured by flow cytometry, followed by manual data analysis and subsequent unsupervised multivariate analysis and data mining by random forest machine learning.

Results: The multivariate algorithm combined with the machine learning tool are capable to stratify the IMID cohorts and the healthy volunteer cohort. Random forest analysis identified several immune subclusters that distingiuish between those disease cohorts. However, many common denominators were found between PS, AD, and IBD.

Conclusion: We developed a semi-supervised analysis pipeline to profile and mine high-dimensional flow cytometry data that might be applied for IMID stratification.

WS.E2E3.01.04

Single cell transcriptomes define the cellular and molecular landscape of human lungs in asthma

F. Vieira Braga1, G. Kan1, T. Gomez1, E. S. Fasoli1, P. Szczeczek1, K. Polanski1, M. Efremova1, K. Mahboubani1, A. Vjechi1, K. Sarb-Parsy1, O. A. Carpini1, M. Berg1, S. Brouwer1, K. Affleck1, M. van den Berge1, A. van Oosterhoud1, M. Nawijn1, S. A. Teichmann1;
1Wellcome Trust Sanger Institute, Cambridge, United Kingdom;
2Cambridge University, Cambridge, United Kingdom;
3University of Groningen, Groningen, Netherlands;
4GlaxoSmithKline, Stevenage, United Kingdom.

The lung plays a critical role in both gas exchange and mucosal immunity. Acute and chronic disorders of the lung are a major cause of mortality worldwide, and some are expressed predominantly in the airways (such as asthma) or in the respiratory unit in lung parenchyma (such as emphysema). We comprehensively profiled the cells that make up both the human lung upper airway wall and the respiratory unit, as well as paired spleens and lymph nodes using single cell transcriptomics. We identified core signatures of lung resident immune cells in healthy individuals. We expanded our analysis beyond healthy individuals by performing single cell transcriptome analysis of whole airways biopsies and sorted CD4 T cells from asthmatic patients. We observed disease specific parameters of epithelial cell differentiation and reshaping of the immune repertoire that takes place in asthma. We reconnected cellular communication network between immune and non immune cells specific for asthma. Our data constitute an invaluable resource for the community interested in immune responses localised to the lung.

WS.E2E3.01.05

DINSE visualization and assessment of clonal kinetics reveals multiple trajectories of dendritic cell development

G. Liu, A. Kan, J. Gao, E. Crampin1, P. Hodgkin1, S. Naoki1;
1Walter and Eliza Hall Institute, Melbourne, Australia;
2University of Melbourne, Melbourne, Australia.

A thorough understanding of cellular development is incumbent on assessing the complexities of fate and kinetics of individual clones within a population. Here we develop a system for robust periodical assessment of lineage outputs of thousands of transient clones and establishment of bona fide cellular trajectories. We appraise the development of dendritic cells (DCs) in fms-like tyrosine kinase 3 ligand culture from barcode-labeled hematopoietic stem and progenitor cells (HSPCs) by serially measuring barcode signatures, and visualise these as multidimensional data using developmental interpolated t-distributed stochastic neighborhood embedding (Di-SNE) time-lapse movies. We identify multiple cellular trajectories of DC development that are characterized by distinct fate bias and expansion kinetics, and determine that these are intrinsically programmed. We demonstrate that conventional DC and plasmacytoid DC trajectories are largely separated already at the HSPC stage. This framework allows systematic evaluation of clonal dynamics and can be applied to other steady-state or perturbed developmental systems.

WS.E2E3.01.06

CD4 T cell transcriptomes reveal novel diagnostic and mechanistic immune signatures of tuberculosis

J. G. Burel, C. S. Undestam Arkehamn1, M. Pomazany1, N. Khan1, G. Seumois1, J. A. Greenbaum1, D. DeSilva1, R. Taplitz1, R. H. Gilmour1, M. Saito1, P. Vijayanand1, A. Sette1, B. Peters1;
1La Jolla Institute for Allergy and Immunology, La Jolla, United States;
2General Sir John Kotelawala Defence University, Colombo, Sri Lanka;
3University of California San Diego, La Jolla, United States;
4Johns Hopkins University Bloomberg School of Public Health, Baltimore, United States;
5Tokyo University, Sendai, Japan.

In the context of infectious diseases, cell population transcriptomics are useful to gain deeper insight into protective immune responses, which is limited using traditional whole blood approaches. As part of the Human Immune Project Consortium (HIPC) program, we are aiming to decipher CD4 T cell immune signatures of tuberculosis (TB) in the context of controlled (latent TB) and not controlled (active TB) infection. We found an ex vivo gene expression signature in memory CD4 T cells that could differentiate between latent TB and TB negative subjects, as well as novel markers that are expressed in TB antigen specific CD4 T cells in the context of latent TB. We further explored the inter-individual variability of gene expression within the latent TB cohort and identified immune parameters associated with higher risk of developing active TB. Finally, comparison of the transcriptomic profile of memory CD4 T cells of latent and active TB subjects after antigen-specific in vitro stimulation identified TB-specific T cell immune signatures of controlled infection versus disease. Overall, our approach has identified a plethora of TB-associated T cell immune signatures that allowed us to gain mechanistic insights into the key CD4 T cell components contributing to TB protective immunity. Additionally, our findings can be translated into new diagnostic and prognostic tools, in particular, identifying latent TB infected individuals at risk of developing active TB.

WS.E4.01

Cell communication and signaling in the immune system

WS.E4.01.01

Mechanotransduction as a novel immune checkpoint in NK cell cytotoxicity

M. Borda-Sood, A. Ben-Shmuel, O. Matalon, J. Kivelitz, B. Sabag, N. Joseph, G. Biber; Bar-Ilan University, Ramat-Gan, Israel.

Natural killer (NK) cells are a potent weapon of the immune system against viral infections and tumor growth. The actomyosin network generates forces through the activity of actin filaments and myosin motors. This machinery is responsible for the conversion of mechanical forces into biochemical signals in a process termed mechanotransduction. However, the way in which mechanotransduction controls the immune response, and specifically lymphocyte activity, is poorly understood. Here, we demonstrate that actomyosin retrograde flow (ARF) controls NK cell response through a novel interaction between beta-actin with the SH2-domain containing protein tyrosine phosphatase-1 (SHP-1), converting its conformation state, thereby regulating NK cell cytotoxicity. Actin dynamics govern SHP-1 conformational structure dictating its catalytic activity. Indeed, blocking actin dynamics results in reduced SHP-1 activity, by confining SHP-1 to its inactivated "closed" conformation. This reduced enzymatic activity of SHP-1 leads to increased phosphorylation of SHP-1 substrates, an elevation of intracellular calcium flux, and NK cell cytotoxicity. Our data suggest that SHP-1 plays a major role as a sensor of ARF-generated forces in the process of mechanotransduction, and reveal a novel mechanism by which regulation of SHP-1 by ARF dictates NK cell killing decisions. Our data identify ARF as a master regulator of the lymphocyte response.
WORKSHOPS

WS.E4.01.02
Combining FACS, High-Resolution Mass-spectrometry, and Single Cell Sequencing Reveals Interactome Effects of Translational Control

Algorithms can target advertisements based on volunteered content and social network in microseconds, yet precision medicine lags in infancy. To break this societal Munchausen by proxy, we generated a framework for immune health by creating a social network of immune cells, modeling both internal state and interactions with other cells. To do this, we analyze and combine data sets including (1) FACS with high-resolution mass-spectrometry-based proteomics and (2) more than 20 single cell whole transcriptome datasets. With (truly) quantitative protein and gene expression data for 28 human hematopoetic cell populations, and more than 10,000 proteins and genes measurements per cell, we generate an interactome for these cells and layer in both transcriptional and translational controls. Using these as internal ‘sentiments’, we develop a deep steady state social network such that perturbations to the internal state of individual cells (e.g. in the context of disease) can be modeled. Using these interactions, (1) CD8 T cell exhaustion, (2) alternate macrophage activation, and (3) dendritic cell dysfunction, we show how perturbations in immune cell gene and protein expression can be monitored, and ultimately treated. Finally, we propose a set of assays to establish a patient steady state profile, and we describe how to build a precision, predictive social network for an individual’s immune system.

WS.E4.01.03
Helper T cell extracellular vesicles couple delivery of effector CD40L to antigen recognition
E. B. Compe1, D. G. Saliba2, F. P. Césedes1, S. Böll1, K. Korobchevskaya1, C. Cassiard1, M. L. Dustin2; 1University of Oxford, Oxford, United Kingdom, 2University of Siena, Siena, Italy.

Most, if not all, cell types release extracellular vesicles (EV) that play a role in local and systemic intercellular communication by transferring protein, lipids and RNA between cells. Exosomes, a subset of EVs, arise through exocytosis of multivesicular bodies that lead to release of their intraluminal vesicles, which are mostly formed by vesicle budding and scission from endosomal membranes by the Endosomal Sorting Complexes Required for Transport (ESCRT). We recently discovered a parallel route of EV generation in CD4 T-cells. We found that >80% of TCR at the immunological synapses’ center, formed with supported planar bilayers (SLB) containing peptide-MHCII and ICAM1, are released from the T-cell surface. These EV thus can be defined as synaptic exosomes (SE) based on budding from the plasma membrane. Mass spectrometry analysis of SE demonstrates the presence of TCR signaling and ESCRT proteins, corroborating our earlier data. While CD40L was not normally detected in SE we could recruit CD40L to SE by incorporating CD40 into SLBs. Super-resolution microscopy on SLBs shows that the majority of SE are TCR positive, with half of the TCR T-EV positive for CD40L, in presence of TCR and CD40 engagement. Exposure of human dendritic cells (DC) to CD40L-SE induced DC maturation. We are investigating the form of CD40L that is stored and translocated in T-cells for packaging and release with TCR at the IS resulting in a feed forward activation of DC with potential for intrinsic store-MHC specificity.

WS.E4.01.04
Itonate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1
D. G. Ryan; Trinity Biomedical Sciences Institute, Dublin, Ireland.

The endogenous metabolite itaconate has recently emerged as a regulator of macrophage function. However, the precise mechanism of itaconate action in macrophages remains poorly understood. Here we report that itaconate is required for the activation of the anti-inflammatory transcription factor nuclear factor erythroid 2 p45-related factor 2 (Nrf2) by LPS. We find that itaconate directly modifies proteins via alkylation of cysteine residues, a novel post-translational modification we’ve termed 2,3-dicarboxypropylation. Itonate alkylates cysteines 151, 257, 273, 288 and 297 on the Kei-like-ECH-Associated Protein 1 (Keap1) enabling Nrf2 to increase expression of downstream genes with antioxidant and anti-inflammatory activity. The activation of Nrf2 is required for the anti-inflammatory action of itaconate. We describe the use of a new cell-permeable itaconate derivative, 4-oxctyl itaconate (OIt), which is protective against LPS-induced lethality in vivo and decreases cytokine production. We show that type I interferons (IFN) boost immunoresponsive gene 1 (Irg1) expression and itaconate production. Furthermore, we find that itaconate production limits the type I IFN response indicating a negative feedback loop involving IFNs and itaconate. Our findings demonstrate that itaconate is a critical anti-inflammatory metabolite acting via Nrf2 to limit inflammation and modulate type I IFNs.

WS.E4.01.05
Sugar sweet signalling: Exploration of dendritic cell lectin receptors and their immune modulatory signalling pathways
R. J. E. Li, E. J. Rodríguez-Comajo, J. Libbets, A. Zaal, C. R. Jimenez, S. R. Piersma, T. V. Pham, R. R. de Goede; de Haas, S. J. van Vliet, Y. van Kooyk; Cancer Center Amsterdam - VU University medical center, Amsterdam, Netherlands.

Dendritic cells (DCs) are key inducers of the adaptive immune response. They possess a multitude of pattern recognition receptors (PPRs), including Toll-like receptors (TLRs), and C-type Lectin Receoters (CLRs) in order to elicit tailor-made immune response against invading pathogens. CLRs have gained much attention as endocytic PPRs, but also for their immune modulatory functions. Strikingly, several carbohydrate ligands are shared among different CLRs, yet each seems to propagate a unique signalling cascade. Additionally, CLR triggering can modulate the signalling pathways of TLRs, to modify or prolong the TLR-induced response.

We recently showed that carbohydrates have a huge impact on DC polarization or suppression of T cell responses. Carbohydrates such as High Mannose-, Lewiss, or Lewisy-containing ligands displayed differential IL-10 and IL-12 expression profiles by DC after concomitant LPS stimulation. In contrast, α2-3- or α2-6-sialic acid-containing ligands skew DCs towards the induction of T reg. These carbohydrates target differential CLRs such as DC-SIGN and Siglec, respectively. To gain further insight the immunogenetic signalling pathways of the DC-expressed CLR DC-SIGN and its interference with TLR signalling, as well as the tolerogenic signalling pathways through Siglec, we coupled different carbohydrate ligands to a dendrimeric structure, thereby offering multivalent ligand presentation. For insight in the underlying signalling pathways, we applied phosphoproteomics to investigate differences in DC protein phosphorylation upon specific CLR-ligand engagement, and next generation sequencing on a transcriptional level. Detailed analysis of these reveal an immunogenic or tolerogenic DC fingerprint through carbohydrate-CLR interaction, and include significant changes in immunogenic signalling pathways.

WS.E4.01.06
DLL4 conveyes Notch-dependent signals achieving selective macrophage polarization or death
B. CHARREAU1, S. Pagie2, A. Pabozi1, N. Géran2, C. Toquet1, P. Huill1, S. Nedellec3; 1CRTI, Nantes, France, 2Plateforme MicroProCIel SFR Santé –IRT, Nantes, France.

1Molecular mechanisms underlying vascular and inflammatory cell network at endothelial and macrophage levels are still unclear. Here we found that microvascular inflammation associates with changes in Notch signaling at endothelial monocyte interface including loss of endothelial Notch4 and the acquisition of the Notch ligand DLL4 in both cell types. We showed that endothelial DLL4 induces circulating monocytes to polarize into a M1-type pro-inflammatory macrophages (CD40+ CD64+ CD200R+ HLA-DR+ CD11b+ eliciting the production of IL-6. DLL4 and IL-6 are both Notch-dependent and are required for macrophage polarization through selective down and upregulation of M2- and M1-type markers, respectively. Subsequently, we investigated the ability of DLL4 to interfere with M2 polarization. We found that DLL4 triggers a specific alteration of the IL-4 induced M2 phenotype through a significant inhibition of M2 markers (CD11b, CD206, CD200R). DLL4 also induces caspase3/7-dependent apoptosis specifically in M2 differentiating macrophages while DLL4 had no effect. DLL4 signals via Notch1 and DLL4-mediated apoptosis is Notch-dependent. Fully differentiated M2 macrophages became resistant to DLL4 action. DLL4 upregulates gene expression, upon M2 upon differentiation, affecting the Notch pattern (Notch1, J. Jag1) and activity (Ihes), transcription (IRFS, STAT1) that associates with decrease in Akt but not STAT6 phosphorylation. In conclusion, our findings reveal an interplay between DLL4/Notch and IL-6/IFN-γ signaling pathways supporting M1 differentiation and impairing M2 differentiation via apoptosis.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 113
POSTER PRESENTATIONS
POSTER PRESENTATIONS

P.A1.01 Myeloid lineage specification - Part 1

P.A1.01.01

Advancing knowledge on human myeloid cell types and activation states through the design and study of in vitro differentiation models.

S. Batail1, C. Arnold-Schrauf2, A. Abbas1, N. Cousegel1, J. Savorot1, F. Imperatore1, A. Villani1, T. Vu Manh1, N. Bharadwaj1, M. Dalod1;

1Aix Marseille Univ, CNRS, INSERM, CIMA, Marseille, France, 2The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, United States, 3Broad Institute of Harvard and MIT, Cambridge, United States, 4Centre d'Immunologie de Marseille-Luminy (IMLM), Marseille, France.

Our understanding of the biology of human dendritic cells and our ability to harness them clinically are hampered by lack of adequate in vitro models of these cells. Ideally, such in vitro models should combine high yields of different types of dendritic cells in the same culture and their amenability to genetic or pharmacological manipulation. To overcome this bottleneck, we developed a method of differentiation of human CD34+ precursors leading to high yields of all the three major types of human blood dendritic cells, namely plasmacytoid dendritic cells, type 1 (CD141-positive) conventional dendritic cells and type 2 (CD1c-positive) conventional dendritic cells. Phenotypic, functional and single cell RNA sequencing analyses were performed to ensure of the identity of the dendritic cell types derived in vitro performed to strom homology with their ex vivo isolated counterparts. This culture system also revealed novel molecular mechanisms governing the differentiation and/or expansion of human dendritic cell types which will be discussed in this presentation. Combining several such recent novel in vitro models for the differentiation of distinct types of human mononuclear phagocytes will greatly facilitate the simultaneous and comprehensive study of primary, otherwise rare, types of human dendritic cells or macrophages, including their mutual interactions.

P.A1.01.02

GM-CSF and M-CSF signaling is integrated at chromatin level during monocyte polarization

R. M. Rodríguez1, B. Suárez Álvarez2, P. Díaz Bulnes2, J. L. Lavín2, A. M. Ascensión2, M. González1, A. Baragaza Raneras1, C. Martin-Martín1, A. Puig-Kräger1, A. L. Corbi1, M. J. Araúzo-Bierno1, A. M. Arana2, C. López Lara2;

1Traslational Immunology Laboratory, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA); Hospital Universitario Central de Asturias (HUCUA); Oviedo, Spain, 2BioGUNE, Bizkaia, Spain, “Computational Biology and Systems Biomedicine Research Group, Biodonostia Health Research Institute, San Sebastían, Spain, “Laboratorio de Inmunometabólismo, Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain, “Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.

Over the last few years, myelopoietic growth factor signaling has emerged as an essential player during monocyte polarization. Due to the apparent antagonism between GM-CSF and M-CSF and their central role during monocyte differentiation, both factors have been proposed as suitable therapeutic targets. Nonetheless, since both cytokines can be simultaneously present in the inflamed tissue it is not clear how signaling is integrated during cell fate decisions or whether monocytes are able to fully reverse their phenotypes in response to apparently conflicting polarization cues. Previous studies showed that a proinflamed chromatin state generated during differentiation or in response to a given stimulus can influence how subsequent activation cues modify gene expression, indicating that the chromatin acts as an integration node during monocyte cell fate decision in the inflammation site. To better understand how undifferentiated monocytes integrate GM/M-CSF signaling at the epigenetic level we performed DNA methylation analysis and ATAC-seq in ex vivo isolated human monocytes. Our results revealed a global and irreversible demethylation trend associated with M-CSF and GM-CSF exposure. In addition, chromatin accessibility analysis showed an extensive epigenetic remodeling after only 12 days exposure in genes typically associated with monocyte polarization. Finally, by specific inhibition of the canonical GM-CSF receptor signaling modules, we were able to dissect the epigenetic changes associated with the JAK-dependent (STAT5, PI3K and MAPK) and JAK-independent signaling pathways. In summary, our results indicate that M-CSF and GM-CSF signaling is rapidly integrated at chromatin level in order to generate stable gene expression patterns during monocyte differentiation.

P.A1.01.03

Identity of human lymphoid organ dendritic cells is predominantly dictated by ontogeny, not tissue microenvironment

G. F. Heidkamp1, J. Sandor1, C. H. Lehmann1, L. Heger1, A. Baranaski1, J. J. Lühr1, A. Hoffmann1, K. C. Reimer1, A. Lux1, A. Hartmann1, T. Ulas1, N. McGovern1, C. Alexiou1, B. Pöppel1, A. Makkerseni, G. Schulr1, R. Repp1, P. A. Fasching1, R. Czemesi1, E. Ullrich1, F. Ginnhub1, A. Schlitz1, F. Nimmenjah1, J. L. Schultze1, D. Udrizki1;

1Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany, 2Hospital of the Goethe University, Frankfurt, Germany.

Dendritic Cells (DCs) are important regulators of immune responses. So far, the distinct functional roles of human tissue DC subpopulations remain largely unknown. In the here presented study, we directly isolated DC subpopulations from various human lympho-hematopoietic tissues (lymph, spleen, bone marrow, tonsils, cord blood, peripheral blood). We investigated whether the percentages of the three major DC subpopulations of CD1c–DCs, CD141+ DCs, and pDCs were varying depending on the tissue analyzed. We next investigated the transcriptional profile of cell sorted DC subpopulations using comprehensive bio-informatic data analyses. Our data are supported by investigation of DC subset localization and functional studies. Overall, we conclude that human DCs of the lympho-hematopoietic system are mainly defined by ontogeny, while non-lympho-hematopoietic DCs (lung, skin) seem to be additionally influenced by modulatory signals from the tissue microenvironment.

The here presented data will help to elucidate the implication of human DC subpopulations in the initiation of immune responses. This work was partly supported by DFG (Emmy-Noether Program, CRC163, CRC1181, CRC045, CRC074, RTG1660, RTG1962, SP1681), Loweer Center, ELAN, IZK, BayGene, and Excellence Cluster ImmunoSensation.

P.A1.01.04

Dendritic cell modulation by mesenchymal stem cell promises a protective microenvironment at the feto-maternal interface: improved outcome of pregnancy in abortion prone mice

M. Eskandarian, A. Yaftian, A. Jahangiri, S. Moszaenni;
Tarbiat modares university, Tehran, Iran, Islamic Republic of.

Introduction: Recurrent spontaneous abortion is one of the most common complications of pregnancy. A major fraction of RSA is related to disorder of the maternal immune system, especially malfunction of dendritic cells (DCs). MSCs have been shown to exert immunomodulatory effects on immune cells especially dendritic cells. The current study investigates whether MSCs are capable to modulate the pattern of maternal immune response via the induction of functional changes in decidual DCs, and finally improves the fetal survival.

Materials and Methods: For this issue, adipose derived mesenchymal stem cells were intraperitonealy administered to abortion prone pregnant mice (CBA/J ×DBA/2) at fourth day of gestation. On day 14.5 of gestation, after determination of abortion rates, the number, phenotype and maturation state of decidural dendritic cells were analyzed using the flow cytometry.

Results: We found that MSCs therapy could decrease the abortion rate significantly and at the same time increases the frequency of decidual DCs. MSCs administration also remarkably decreased the expression of MHC-II, CD86 and CD40 markers on decidual DCs in MSC-treated group. In contrast, CD11b significantly increased in these group compared to non-treated mice.

Conclusions: Our results indicated that MSCs could change the microenvironment of decidua through secretion of various components or direct cell-cell contact and correct the immune cell disorders by modulating the DCs function.

P.A1.01.05

Gene scan analysis by detection of GTdel in exon 2in NCF1 gene for AR-CGD with p47 defect

M. Y. Koker, B. Saraymem;
Erçyes Medical school, Kayseri, Turkey.

CGD patients have 5 different gene which is most popular for genetic mutations. But in patients with residual oxidative activity in DHR assay mostly have mutation in NCF1 gene defect result in p47-phox defect. Most of mutation in CGD and NCF1 gene are result from hot-spot mutation(C.75_76delGT) in exon which results in p.Try26HisFx26. One of the fastest method for detection of mutation in these patients are gene scan analysis by using microstellite analysis for detection of pseudogene/gene ratio in NCF1 gene. When the gene scan peak ratio is 1, this usually means that one allele there is the normal configuration of two pseudogenes and one NCF1 gene. On the other allele we then find one pseudogene, one NCF1 gene and one fusion gene of the 5’part NCF1 gene and the 3’part pseudogene. The gene scan detects the 5’part of the pseudogenes and the NCF1 gene. That leads us to use three genes with the defects (pseudogene) and three genes with the GTGT configuration (like in NCF1 genes). Thus, the ratio is 3:3 = 1:1. When the fusion genes are inactive, so you can regards this as a heterozygote for NCF1 deltAGT. In such a person, there is a no defect in oxidative activity, p47 expression or microbicalid activity. We have dizany a gene-scan diagram first time in the literature.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 115
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.A1.01.06
Regenerative macrophages in the skin interconnect immune and nervous system

J. Kalter1, R. Feuerstein2, N. Hagemeier1, P. Zeis1, N. Patersoi1, D. Grün1, T. Lämmermann1, M. Prinz3, P. Henneke4.
1Center for Chronic Immunodeficiency, Freiburg, Germany, 2Faculty of Biology, University of Freiburg, Freiburg, Germany, 3Institute of Neuropathology, Freiburg, Germany, 4Max-Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany.

Since dermal macrophages but not monocytes are central for the defense against Staphylococcus aureus, we reasoned that heterogeneous mechanisms of macrophage replacement coexist in the skin. Here, we scrutinized the origin of dermal macrophages and a potential postnatal specification into subpopulations by utilization of reporter mice, fate-mapping strategies and transcriptomics. We found that dermal macrophages underwent fundamental changes after birth. While the population initially uniformly expressed the fractalkine receptor at high level (CX3CR1+), only a small fraction remained positive in adult mice. CX3CR1+ macrophages were only slowly replaced after bone marrow transfer and exhibited long persistence after tamoxifen-dependent fate mapping in CX3CR1-loxP/loxP;R26-YFP mice. In contrast, other dermal macrophages were short-lived and received major bone-marrow input. Single cell RNA sequencing revealed six major macrophage clusters in the skin of which one corresponded to the CX3CR1+ population. The respective subset closely and exclusively colocalized to sensory nerve axons in the dermis of adult mice and showed a distinct expression signature with over 500 upregulated genes including anti-apoptotic and migratory markers in bulk transcriptomic analysis. Specific expression markers of connections to the nervous system along with bidirectional migration along nerve axons and uptake of myelin point towards a role interconnecting immune and nervous system, e.g. in the context of inflammatory nerve damage. Collectively, these data point to a sub-division of skin macrophages leading to heterogeneity in phenotype, function and origin within the very same tissue.

P.A1.01.07
Defining the role of E-cadherin in hematopoiesis

R. A. Krimpenfort, M. Nethe; Sanquin Blood Supply, Amsterdam, Netherlands.

During erythropoiesis red blood cells (RBCs) are generated to adsorb and transport oxygen which is a fundamental process to sustain life. The generation of RBC’s importantly relies on stem cells in the bone marrow which produce RBCs during erythropoiesis. Mice4, 5 expressing a fate-mapping strategy for primitive hematopoietic progenitors in vivo5, we were able to show that primitive hematopoiesis is characterized by CR11bright macrophages which were only slowly replaced after bone marrow transfer and exhibited long persistence after fate mapping in CR11-loxP/loxP;R26-yfp mice. In contrast, other hematopoietic progenitors were short-lived and received major bone-marrow input. Single cell RNA sequencing revealed six major macrophage clusters in the skin of which one corresponded to the CR11+ population. The respective subset closely and exclusively colocalized to sensory nerve axons in the dermis of adult mice and showed a distinct expression signature with over 500 upregulated genes including anti-apoptotic and migratory markers in bulk transcriptomic analysis. Specific expression markers of connections to the nervous system along with bidirectional migration along nerve axons and uptake of myelin point towards a role interconnecting immune and nervous system, e.g. in the context of inflammatory nerve damage. Collectively, these data point to a sub-division of skin macrophages leading to heterogeneity in phenotype, function and origin within the very same tissue.

P.A1.01.09
Selective expansion of lineage-primed progenitors guides emergency haematopoiesis

D. Lin, P. Hodgkin, S. Nalls; Walter and Eliza Hall Institute, Melbourne, Australia.

Single hematopoietic stem and progenitor cells (HSPCs) are demonstrated to be lineage-primed in the steady state using clonal fate tracking and single cell RNA profiling. However, when single cell fate is regulated during emergency hematopoiesis, which is the processes of cytokine induced or acute injury induced expansion of lineage-primed HSPCs, it is still unclear how these different types of lineage-primed HSPCs contribute to hematopoiesis. To characterize the role of lineage-primed HSPCs during emergency hematopoiesis, we used a novel mouse model which allows somatic inactivation of E-cadherin in the hematopoietic cell lineage.

P.A1.01.11
Investigation of BATF-dependent gene expression signatures in plasmacytoid dendritic cells

R. Mann-Nütte1, S. Alpi1, P. Petzsch1, K. Köhre2, H. Xu3, P. Long3, S. Scheu1.
1Institute of Medical Microbiology and Hospital Hygiene, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, 2Cluster of Excellence EXC 1003, Cells in Motion, University of Münster, Münster, Germany, 3Biomedical Research Facility, Heinrich Heine University Düsseldorf, Düsseldorf, Germany.

Plasmacytoid dendritic cells (pDCs) are known to produce large amounts of type I interferon (IFN) during viral infections. Contrasting the previous dogma, we found that only a small subset of pDCs produces type I IFN after TLR9 activation. Microarray experiments revealed that the transcription factor Batf (basic leucine zipper ATF-like transcription factor) is highly expressed in these type I IFN-producing pDCs. While the function of BATF for T helper cell subset differentiation and B cell class switching has been well described, we show that BATF is also essential for pDC differentiation. pDCs from Batf-/- mice are not able to produce type I IFN after TLR9 activation. These data suggest that BATF is critical for pDC development and may play a role in the development of type I IFN producing pDCs. Further, we are currently investigating whether BATF has a role in the development of type I IFN-producing pDCs in the absence of BATF.

P.A1.01.12
Developmental heterogeneity of early life dendritic cells

N. E. Papaioannou1, J. Salvermoser1, N. Salei1, M. Ptasz1, R. Schuchert1, S. E. W. Jacobsen1, C. Schulz1, U. B. Schraml1,2.
1Biomedical Center, LMU Munich, Planegg-Martinsried, Germany, 2Walter-Brendel-Centre for Experimental Medicine, University Hospital, LMU Munich, Planegg-Martinsried, Germany.

Dendritic cells (DCs) are immune sentinel cells that initiate, polarize and orchestrate adaptive and innate immune responses. Early life DCs differ both quantitatively and qualitatively from those in adults. Neonatal DCs, for instance, show drastically reduced immune responses less efficiently than their adult counterparts, because they trigger reduced T cell proliferation. Additionally, they produce low levels of immunostimulatory cytokines resulting in Th2 biased immune responses. As a consequence, neonates are more susceptible to infection with certain pathogens than adults.

Using a model for fate mapping of conventional DC precursors (CDP) with yellow fluorescent protein (YFP), we have revisited the development of DCs during mouse embryogenesis, as well as in perinatal and adult mice. Surprisingly, we find that CD11c+MHCII+CD11b+ splenocytes, which phenotypically resemble CDP-derived CD11b+ DCs that label strongly with YFP in adult mice, were poorly labeled in embryonic and neonatal mice. Combined with additional fate mapping experiments for primitive and definitive hematopoietic progenitors in vitro, we suggest that DCs exhibit age-dependent heterogeneity in terms of their ontogeny. Currently, we are investigating the impact of this developmental heterogeneity on the capacity of early life DCs to respond to pathogenic stimuli and initiate immune responses. Taken together, we show that a layered DC development may contribute to the qualitatively altered DC responses in early life. This work is supported by an ERC Starting Grant awarded to BS (ERC-2016-STG-715182).
POSTER PRESENTATIONS

P.A1.01.13 Therapeutic targeting of hexosamine biosynthetic pathway in acute myeloid leukemia

R. Parameswaran, A. Ashkan
CASE WESTERN RESERVE UNIVERSITY, CLEVELAND, United States.

Acute myeloid leukemia (AML) is the most common acute leukemia among adults with an overall poor prognosis. For 40 years there has been minimal improvement in treatment beyond induction chemotherapy or allogeneic stem cell transplantation. Targeting cell metabolism is a promising avenue for future cancer therapy. We found that enzymes involved in metabolic hexosamine biosynthetic pathway (HBP) is increased in AML patients. The HBP metabolizes glucose and glutamine to produce UDP-N-acetylglucosamine (UDP-GlcNAc) which is the substrate for O-GlcNAcylation, a post translational modification on cytosolic and nuclear proteins. We found that glutamine-fructose-6-phosphate-amineotransferase (GFAF), the rate limiting enzyme of HBP pathway and D-GlcNAc transferase (OGT), which catalyzes the addition of GlcNAc to proteins are significantly increased in different AML patient subtypes as well as different AML cell lines. Inhibiting GAFAT pathway can kill AML cells by triggering cell cycle arrest, inhibits cell proliferation and finally leads to apoptotic cell death, sparing normal cells. Knockdown of OGT enzyme using OGT shRNA also led to AML cell differentiation and cell death. Finally, targeting of HBF in vivo leads to significant clearance of tumor cells in an AML xenograft mouse model, with minimum toxicity. This study reveals an important role of HBF in keeping AML cells in the un-differentiated and malignant state and sheds light into a new area of potential AML therapy by targeting HBF pathway.

P.A1.01.14 Mesoporous Silicon Microparticles enhance inflammatory responses in human macrophages

I. Real Arévalo1,2, B. Amorós-Pérez1, A. Revilla1,2, L. Diego-González1, B. Martín-Adorés1, R. Martín-Palma1, E. Martínez-Naves1, M. Gómez del Moral2
1Departamento de Inmunología. Facultad de Medicina. Universidad Complutense, Madrid, Spain, 2Departamento de Biología Celular. Facultad de Medicina. Universidad Complutense, Madrid, Spain, Departamento de Física Aplicada, Universidad Autónoma de Madrid, Madrid, Spain.

Introduction: Mesoporous silicon microparticles (MSMPs) possess unique chemical stability, adjustable pore size, extensive surface area, biocompatible and biodegradable nature, that offer large advantages over current adjuvants or vehicles. We have previously described that peptide-loaded MSMPs enhance antigen specific T-cell activation by human monocyte derived dendritic cells. Macrophages are important APCs whose activity can be polarized to a proinflammatory (M1) or anti-inflammatory (M2) phenotype. Aim: Our goal was to investigate how macrophages respond to MSMPs.

Methods: MSMPs were fabricated by electrochemical treatment of silicon wafer. M1 or M2 Macrophages were obtained after 7 days of incubation with GM-CSF or M-CSF respectively, from CD14+ monocytes isolated from Peripheral blood mononuclear cells (PBMCs) using immunomagnetic microbeads. M1 and M2 phenotype was assessed by gene and phenotype expression by quantitative real-time PCR and flow cytometry respectively. Cytokines were quantified in culture supernatants by ELISA. Endocytosis was assessed by flow cytometry using FITC labeled MSMPs.

Results: Our results showed that MSMPs are efficiently endocyted by both M1 and M2 polarized macrophages, in a process that is in part mediated by scavenger receptors. Enhanced CD80, CD86, HLA-DR and CD16 markers on the cell surface in both M1 and M2 macrophages were observed after MSMP endocytosis. There was an increase of TNFα, IL6 and IL12 secretion in macrophages treated with MSMPs. No production of lL-10 was observed in M1 or M2 cell sarubants.

Conclusions: Mesoparticles of silicon monoxide are endocyted by macrophages favouring a proinflammatory profile (M1) and inhibiting the IL-10 production.

P.A1.01.15 Developmental origin and cellular identity of cardiac macrophages in steady state and in response to stress

C. Schulz, T. Weinberger, V. Schneider, E. Gomez Perdiguero
Ludwig Maximilians University, Munich, Germany, Institut Pasteur, Paris, France.

Background: Macrophages are the most prominent immune cells in myocardial tissue and play a critical role in pathological conditions. However, the quantitative contribution of embryonic and bone marrow (BM) hematopoiesis to the cardiac macrophage pool under steady state and in response to stress has been unknown. Methods/Results: In this study we mapped the origin and fate of the different macrophage lineages in mouse models of ischemia-reperfusion injury (I/R) and Angiotensin II-induced fibrosis (AT-II). Using FLt3-Cre mice and radiation-independent BM chimera, we found that under steady state a considerable amount of cardiac macrophages develops independently of definitive hematopoiesis. In response to both I/R and AT-II, we observed an increase in the number of BM-derived macrophages in the heart. However, this increase was only transient and the number of BM-derived macrophages declined over time reaching steady state numbers after 30 days. After AT-II infusion, we found an increase of BM-derived macrophages in areas of myocardial and interstitial fibrosis, whereas after I/R-injury there was a profound influx of BM-derived macrophages in the infarct region and its adjacent remote area. The influx of BM-derived macrophages could be significantly reduced by deletion of CCR2. Conclusion: Embryonic macrophages are the major contributors to the pool of cardiac tissue-resident macrophages in adult mice. In the acute phase of inflammation, BM macrophages invade the heart but do not persist in significant numbers in myocardial tissue. Our findings are of potential relevance for understanding the cardiac immune response and for the therapeutic targeting of macrophages in inflammatory conditions.

P.A1.01.16 TCDD-mediated activation of aryl hydrocarbon receptor alters microRNAs (miRs) expression in Granulocytic Myeloid Derived Suppressor cells (G-MDSCs) and Splenic Resident Granulocytes (SRG) via shared and unique pathways of differentiation

N. P. Singh, D. Jackson, U. P. Singh, S. Sumpter, P. Nagar Karti, M. Nagar Karti; University of South Carolina School of Medicine, Columbia, United States.

TCDD is an AhR ligand and has potent immunosuppressive effects on humans and animals. Recently, we have shown that TCDD triggers induction of myeloid derived suppressor cells (MDSCs) in mouse models of ischemia-reperfusion injury (I/R) and Angiotensin II-induced fibrosis (AT-II). Using FLt3-Cre mice and radiation-independent BM chimera, we found that under steady state a considerable amount of cardiac macrophages develops independently of definitive hematopoiesis. In response to both I/R and AT-II, we observed an increase in the number of BM-derived macrophages in the heart. However, this increase was only transient and the number of BM-derived macrophages declined over time reaching steady state numbers after 30 days. After AT-II infusion, we found an increase of BM-derived macrophages in areas of myocardial and interstitial fibrosis, whereas after I/R-injury there was a profound influx of BM-derived macrophages in the infarct region and its adjacent remote area. The influx of BM-derived macrophages could be significantly reduced by deletion of CCR2. Conclusion: Embryonic macrophages are the major contributors to the pool of cardiac tissue-resident macrophages in adult mice. In the acute phase of inflammation, BM macrophages invade the heart but do not persist in significant numbers in myocardial tissue. Our findings are of potential relevance for understanding the cardiac immune response and for the therapeutic targeting of macrophages in inflammatory conditions.

P.A1.01.17 Precise delineation and transcriptional characterization of bovine dendritic-cell and monocyte subsets

S. C. Talker1,2, A. Baumann1,2, G. T. Barut1,2, I. Keller1, R. Bruggmann1, A. Summerrfield1,2
1Institute of Virology and Immunology, Bern and Mittellansädten, Switzerland, 2Department of Infectious Diseases and Pathobiology, Vetwsiss University, Bern, Bern, Switzerland, Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, Bern, Switzerland.

A clear-cut delineation of bovine bona fide dendritic cells (DC) from monocytes has proved challenging, given the high phenotypic and functional plasticity of these immune cells and the marked phenotypic differences between species. Here, we demonstrate that, based on their expression of Flt3, CD172a, CD13, and CD4, a precise identification of bovine blood conventional DC type 1 and 2 (cDC1, cDC2), plasmacytoid DC (pDC), and monocytes is possible with CD1c being Flt3-CD172a-CD13+CD4+CD2c being Flt3-CD172a-CD13+CD4- pDC being Flt3-CD172a-CD13+CD4- and monocytes being Flt3-CD172a+CD13+CD4+. The phenotype of these subsets was characterized in further detail, and a subset-specific differential expression of CD2, CD5, CD11b, CD11c, CD14, CD16, CD40, CD71, CD163, CD205, and CD11b1 was found. Subset identification was enabled by flow cytometry and subset-specific expression of conserved key genes. We also sorted monocyte subsets based on their differential expression of CD14 and CD16. Classical monocytes (CD14+CD16-) clustered clearly apart from the two CD16+ monocyte subsets representing intermediate and non-classical monocytes described in human. The transcriptomic data also revealed differential expression of molecules involved in antigen presentation, pathogen sensing, and migration, and therefore gives insights into functional differences between bovine DC and monocyte subsets. The finding that antigen-specific and subset-specific cell expression will assist in the development of “bovine marker molecules” that – when targeted by flow cytometry – will greatly facilitate research on bovine DC and monocytes. Overall, species comparisons will elucidate basic principles of DC and monocyte biology and will finally help to translate experimental findings from one species to another.
Role of serum Hypoxia-Inducible Factor (HIF)-1a in patients with COPD

A. Eremin, R. Ivanek, C. Jansen, D. Hume

Introduction: HIF-1α protein is a master transcriptional regulator of the adaptive response to hypoxia which increases with initiation of oxidative stress. However, the significance of this protein in COPD patients with smoking habit is still unknown.

Method: In this case controlled study, we enrolled 57 COPD patients, along with 15 smokers without COPD and 15 healthy individuals (as control sets). The mean age of patients was 54.6 ± 9.32 years and controls 50.0 ± 9.8. The study set included 62% smokers, 25% non-smokers, 7% tobacco chewers and 6% ex-smokers. Sandwich Enzyme-linked Immuno Sorbent Assay (ELISA) method was used for analysing serum HIF-1α levels.

Results: In all patients with smoking habit, a positive association with hypoxia, smoking status and severity of disease (p<0.03). However, the mean value of HIF-1α was not significantly different in smokers without COPD and healthy controls.

Conclusion: Our study suggests positive association of HIF-1α levels with smoking habit of COPD patient and severity of the disease unlike the smokers without COPD suggesting that activation of HIF-1α pathway in COPD.

Evolutionary conservation of bone marrow-derived antigen presenting cells

R. van den Bieglma, V. Rutten1, W. van Eden, E. Jansen

1Utrecht University, Utrecht, Netherlands, 2Pretoria University, Pretoria, South Africa.

Dendritic cells (DCs) are antigen presenting cells (APCs) that bridge the innate and the adaptive immune system. Many studies on DCs are based on in vitro differentiation of human monocyte- or murine bone marrow-derived progenitor cells into APCs in the presence of GM-CSF and IL-4. Whether these cells resemble their in vivo counterpart is still under debate. Recent culture methods have been developed to generate chicken bone marrow-derived APCs. We aim to characterize this heterogeneous cell population in chickens and to study “evolutionary conservation” of APCs in this non-mammalian species. Cells isolated from femurs and tibiae of chicken embryos were cultured in the presence of recombinant chicken IL-4 and GM-CSF. At day 7, these immature APCs were matured by LPS stimulation. Flow cytometry, fluorescent microscopy, and RT-qPCR were performed to characterize these cells. The majority of the cells are CD11b/c+CD206 (MRC1)+CD117 (cKit)+CD40+. Within this population, half of the cells are MHCII+CD80+CD115 (CSF1R)+ and resemble mammalian DCs. The other cells are MHCII+CD80+CD115 (CSF1R)+ and resemble macrophage-like cells. The latter are larger in size and have a higher phagocytosis capacity than MHCII+ cells. Similar subpopulations have been observed in murine bone marrow-derived DCs. However, murine macrophage-like MHCII− cells are known to lack expression of CD40 or ckit, in contrast to the MHCII+ subpopulation that we have observed. Despite these differences, murine and avian bone marrow-derived APCs show high similarity, suggesting an evolutionary conservation of APC marker expression and potentially their role within the immune response in mammalian and non-mammalian species.

TIE2 Protein Specifies the Functional Polarization of Myeloid-derived Suppressor Cells during Tumorigenesis

D. Yan, L. Wang1, M. Xu1, O. Adeleye, Y. Chen1, X. Wan1

1Chinese Academy of Sciences, Shenzhen, China, 2University of Pennsylvania, Philadelphia, United States.

Myeloid-suppressor cells (MDScs) are “polarized” myeloid cells that effectively promote tumorigenesis by inhibiting anti-tumor immunity. How myeloid cells acquire the pro-tumor properties during tumorigenesis and what specifies their functional phenotype is still unclear. Here we report that the pro-tumor phenotype of myeloid cells is induced by the bivalent growth factor (BGF) pro-tumor ligand TIE2 (tumor necrosis factor-a-induced protein 8-like 2) that mediates the functional polarization of MDScs by specifying their pro- and anti-tumour properties. Tumor cells induce the expression of TIE2 in GD1+CD11b+ cells through reactive oxygen species (ROS). TIE2 in turn is expressed in the pro-tumoral mediators such as CCAAT/enhancer-binding protein-β while inhibited the expression of anti-tumoral mediators. Consequently, tumor growth in TIE2 deficient mice was significantly reduced, and TIE2-deficient MDScs markedly inhibited tumor growth upon adoptive transfer. Pharmacological blockade of ROS inhibited TIE2 expression in MDScs and reduced tumor growth in mice. These findings indicate that TIE2 plays a key role in the functional polarization of MDScs and represents a new therapeutic target for cancer immunotherapy.

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Recent evidence highlights that mitochondrial dynamics orchestrate macrophages metabolism and polarization.
Taking advantage of flow cytometric techniques and co-culture with 3D organoids, we show that PRRs ligation by microbial products is able to drive human HSPCs differentiation. Thus, we aim to unravel the molecular mechanisms underlying this process by analysing the role of downstream signalling and the activation of specific genetic programs in a human model of hematopoiesis.

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P.A1.02.06
Altered immune responses in mice with LST1 adaptor deficiency

M. Fabisik1, J. Králová1, J. Jurečková1, S. Borna1, T. Skupová1, J. Procházka1, F. Spoušek1, B. Malásková1, R. Sedlacek1, T. Brdička1
1Institute of Molecular Genetics of the ASCR, v. v. i., Prague, Czech Republic, 2CEZ Centre for Phenogenomics, hosted by the Institute of Molecular Genetics ASCR, Prague, Czech Republic, 3Centre for Microscopy and Molecular Imaging, Aix-Marseille Université, INSERM, CNRS UMR, Marseille, France.

Transmembrane adaptor protein LST1 is expressed in leukocytes of the myeloid lineage. Previous study has revealed mild effects of LST1 deficiency on the outcome of influenza infection in mice. Except for this specific case, its overall function at the organ level is still to be determined. At the molecular level, LST1 was shown to interact with cytoskeleton regulating proteins and to promote the formation of tunneling nanotubes. It also contains an ITIM motif in its intracellular tail, which was shown to bind phosphatases. In this study, we focused on the role of LST1 in immune responses, as we recently reported that LST1 is required for leukocyte development and immune system homeostasis, but it is involved in the regulation of several types of immune responses. Financed by GACR P302/12/G101.

P.A1.02.07
Experimental human endotoxemia results in accelerated maturation of the neutrophil compartment in the human bone marrow

E. van Grinsven1,2, S. van Staveren3, M. Hassoni4, K. Tesselaar5, G. Leijte6, M. Kaš7, P. Pickkers8, N. Viskeapo9, L. Koenderman1;
1Laboratory of translational immunology, Utrecht, Netherlands, 2University Medical Center Utrecht, Utrecht, Netherlands, 3Radboud University Medical Center, Nijmegen, Netherlands.

Neutrophils are thought to be short-lived cells and are produced in extremely high numbers in the bone marrow (>10^11 cells/day). Nevertheless, solid data to support this hypothesis is lacking. We investigated the proliferation and maturation of the neutrophil compartment in humans during homeostasis and experimental human endotoxemia, as in vivo model for acute systemic inflammation.

Bone marrow and blood were obtained from healthy volunteers before and during acute septicemia induced by intravenous challenge with endotoxin (lipopolysaccharide, 3ng/kg). By differential CD11b/CD11c staining promyelocytes, myelocytes, metamyelocytes, banded cells and mature cells were identified and sorted for analysis on cytospins. Differentiation was also studied with a phenotyping panel, containing Ab against CD305, CD49d, CD16, CD62L, CD11b, CD35, CD66b, CD11c, CD13, and CD64.

Four hours after endotoxin-challenge, the neutrophil compartment exhibited signs of accelerated maturation, characterized by an increased number of cells with more mature characteristics in all neutrophil fractions. Although large numbers of banded neutrophils were found in the peripheral blood, these cells in the bone marrow did not decrease. Also, the number of CD62L+ neutrophils increased in both blood and bone marrow.

In conclusion, our study shows that the neutrophil compartment in the bone marrow of healthy volunteers responds immediately to systemic inflammatory cues and shows signs of accelerated maturation. During accelerated inflammation, the release of neutrophils with banded or hypersegmented nuclei does not lead to depletive of these cells in the bone marrow.

P.A1.02.08
Multimetric in situ characterisation of macrophages in gastric cancer

Y. Huang1,2, M. Wang1, Y. Sun1, N. Di Costanzo1,2, C. Mitchell1, A. Achuthan1, R. Busuttil2, J. Hamiliton1, A. Boussigot1,2,3
1Upper Gastrointestinal Translational Research Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia, 2Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Australia, 3Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia.

Introduction: Tumour-associated macrophages (TAMs) are one of the most important components of the microenvironment in gastric cancer (GC) and have been shown to exhibit immune suppressive and tissue remodelling properties. The M1/M2 system is commonly used to classify TAMs but may represent an oversimplification of a continuum of macrophage phenotypes. Despite intense characterisation of in vitro polarised macrophages, their heterogeneity in situ is not well understood. We performed a comprehensive study to identify and characterise the macrophage populations in the GC microenvironment and to determine their relevance to clinical outcome. Materials and Methods: A macrophage specific seven-colour immunohistochemistry panel was applied to 56 formalin-fixed paraffin-embedded GC patient specimens. Multiple images of each specimen were analysed to account for tumour heterogeneity. Samples were phenotyped using the inForm software (PerkinElmer). Cell number and spatial distribution were analysed with respect to tumour phenotype. However, when we challenged LST1-deficient mice with pro-inflammatory stimuli some aspects of their responses were altered. Intraportal injection of viral mimetic PolyIC resulted in significant reduction in splenic CD8+ T cell percentages. However, the most striking differences were observed when we induced acute colitis in these mice by dextran sodium sulphate, as a model of disease, where myeloid cells are heavily involved. We found significantly better course of acute colitis in LST1-deficient animals in all observed parameters. This was accompanied by alterations in splenic monocyte populations. Interestingly, we also saw the same significant decrease in CD8+ splenic T cells as after polyIC injection. Collectively our data suggest, that LST1 is not required for leukocyte development and immune system homeostasis, but it is involved in the regulation of several types of immune responses. Financed by GACR P302/12/G101.

CD11b Expression in CGD patients and its use in X-CGD carrier detection

M. Y. Baker, B. Saraymen, E. Bentli
Erçyes medical school, Kayseri, Turkey.

Cytokine-granuloma disease (CGD) is a rare primary immunodeficiency disorder of phagocytes resulting in impaired killing of bacteria and fungi. X-linked CGD have been reported to be responsible for approximately 65% of all CGD cases and AR-CGD is 35% of them. The CD11b is known as the integrin aM subunit. CD11b is highly expressed on neutrophils, monocytes and macrophages. Oxidative burst activity and the expression of CD11b, CD18 on neutrophils, monocytes and macrophages. Oxidative burst activity and the expression of CD11b, CD18 has been used as indicators of leukocyte activation status. CD11b, CD18 integrins on the PMN membrane not only mediate cell adhesion, but they also mark for activation of protein tyrosine kinases. Neutrophil function were controlled with DHR assay in 24 samples 8 controls, 8 X-CGD patient, 8 X-CGD Carrier. Than, this assay were done after stimulation of (PMA), the expression CD11b on neutrophil was measured by flow cytometric analysis. The results were compared with DHR 123 activation of protein tyrosine kinases. Neutrophil function were controlled with DHR assay in 24 samples 8 controls, 8 X-CGD patient, 8 X-CGD Carrier. Than, this assay were done after activation of protein tyrosine kinases. The M1/M2 system is commonly used to classify TAMs but may represent an oversimplification of a continuum of macrophage phenotypes. Despite intense characterisation of in vitro polarised macrophages, their heterogeneity in situ is not well understood. We performed a comprehensive study to identify and characterise the macrophage populations in the GC microenvironment and to determine their relevance to clinical outcome. Materials and Methods: A macrophage specific seven-colour immunohistochemistry panel was applied to 56 formalin-fixed paraffin-embedded GC patient specimens. Multiple images of each specimen were analysed to account for tumour heterogeneity. Samples were phenotyped using the inForm software (PerkinElmer). Cell number and spatial distribution were analysed with respect to tumour phenotype.

P.A1.02.09
MDM2 promotes cell cycle arrest and predicts poor patient outcome in gastric cancer

H. C. Reep1,2,3
1Laboratory of translational immunology, Utrecht, Netherlands, 2University Medical Center Utrecht, Utrecht, Netherlands, 3Radboud University Medical Center, Nijmegen, Netherlands.

Introduction: The CD163+ (CD206-) TAMs were associated with improved survival in patients where these macrophages were both abundant in the tumour core and in direct contact (10μm) with the tumour cells. Conclusions: Macrophages are heterogeneous even within the same tissue. Spatially resolving their density and co-localisation with the tumour cells could help understand their interactions, provide better survival predictions and identify possible therapeutic candidates for GC patients.

P.A1.02.10
Ontogeny of tissue-resident macrophages during blood-stage malaria

S. Lat1, C. Rod2
1School of Biological Sciences, Nanyang Technological University, Singapore, Singapore, 2Singapore Immunology Network, A*STAR, Singapore, Singapore.

Blood-stage malaria potently initiates both innate and adaptive immune responses, inclusive of the activation and modulation of the mononuclear phagocyte network. Plasmodium infection results in a profound loss of embryonically established tissue-resident macrophages primarily in the spleen, heavily involving the liver and lungs; and whose numbers are restored following clearance of Plasmodium parasites. During acute blood-stage malaria, both self-renewing tissue-resident macrophages and bone marrow monocytes contribute to the repopulation of the emptied niches of splenic red-pulp macrophages and liver Kupffer cells, unlike lung alveolar macrophages that are capable of refilling their niche mainly through self-renewal. Despite Plasmodium infection, the liver allows the replenishment of macrophages to gain almost identical gene expression even within the same tissue. Spatially resolving their density and co-localisation with the tumour cells could help understand their interactions, provide better survival predictions and identify possible therapeutic candidates for GC patients.
Myeloid-Derived Suppressor Cells originate from bone marrow and are induced by G-CSF and persist in septic shock

E. Leceta1, 2, T. Daic, 3, E. Guerin1, 3, B. Francois1, 3, J. Feuillard1, 3, S. Alain, 3, R. Jeannet, 3

1UMR CNRS 7276, Medicine University, Limoges, France, 2UMR Inserm 1092, Medicine University, Limoges, France, 3Medical-surgical ICU, Dupuytren Teaching Hospital, Limoges, France, 4Hematology Laboratory, Dupuytren Teaching Hospital, Limoges, France.

Introduction: Myeloid-derived suppressor cells (MDSC) are composed by a polymorphonuclear (PMN-MDSC) and a monocytic (M-MDSC) subset. Their functions are well described in cancer but it remains unclear how they are produced and recruited during sepsis. Methods: 57 immunocompetent patients hospitalized in Limoges Hospital for an acute infection were enrolled and regrouped according to their clinical severity: infection, sepsis and septic shock. We followed the level of M-MDSC and PMN-MDSC by flow cytometry in Peripheral Blood (PB) at D0, D3, D7 and D14. Plasma was collected to perform pro-inflammatory cytokines analysis. 20 residual Bone Marrow (BM) samples for thrombocytopenia diagnosis at admission were available for MDSC analysis. BM without hematological diseases were used for cultured assay. Results: When compared to healthy controls at admission, all patients showed an increase of MDSC absolute number in PB regardless of severity. Similarly, cytokine storm is visible in all patients, but cytokine decrease is slower in the most severe patients. MDSC were functional in vitro and normal BM were able to produce M-MDSC and PMN-MDSC in presence of G-CSF and GM-CSF. Finally, MDSC are present in the BM of septic and septic shock patients with thrombocytopenia. Conclusion: We demonstrated that MDSC are produced by the BM following an infection and released rapidly in the PB. Interestingly, a sustain or increase of MDSCS after 3 days of hospitalization is linked to the severity of the sepsis and cytokine level in PB. Survival analyses are under investigation.

Identification of monocytes from Asian elephant (Elephas maximus) by flow cytometry confirms a large population of PBMC with a diverse morphological profile

T. Maeha1, A. Dastjerdi2, J. Lopez,4, F. Steinbach1, 5

1Institute of Veterinary Medicine, Leibniz- Institut, United Kingdom, 2Animal and Plant Health Agency (APHA), Weybridge, United Kingdom, 3Chester Zoo, Upton by Chester, United Kingdom.

Asian elephants (Elephas maximus) are a critically endangered species where captive and wild populations threatened by a range of pathogens such as Elephant endotheliotropic herpesviruses (EEHV). EEHV can cause a fatal haemorrhagic disease (HD) in juveniles and the inability to culture the virus in combination with a lack of understanding of the elephant’s immune system has hampered the development of effective prophylactics. Specifically, only a few reagents are available for the analysis of the elephants’ immunity, which may play a critical role in containing herpesvirus infections. We assessed a panel of commercially available monoclonal antibodies (mAbs) for cross-reactivity and suitability to characterise elephant subtypes. Initial flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) revealed a forward and side scatter (FSC/SSC) pattern that is different when compared to other species, indicating a large putative monocyte population (>30%). This was corroborated by the staining pattern of an anti-bovine CD14 mAb found to have inter-species reactivity with elephant PBMCs whereby CD14+ cells could be visualised as two potential monocyte subsets in FSC/SSC plots. In addition, an anti-MHCII, an anti-CD3 and a mAb against CD8 cells react with elephant leukocytes. Together these Ab’s indicate an unusual composition of immune cells in Asian elephant that requires further investigation into the correlation with age and to better understand how monocytes dominate the PBMCs.

Functional alterations in monocytes derived from bone marrow of myeloid dysplasia syndrome

L. B. Camero1, N. S. Baco2, L. Morti3

1Hospital Israelita Albert Einstein - Clinical Pathology Laboratory, São Paulo, Brazil, 2Centro de Hematologia de São Paulo and Hospital Israelita Albert Einstein, São Paulo, Brazil, 3Hospital Israelita Albert Einstein - Clinical Pathology Laboratory, São Paulo, Brazil.

Background: Myelodysplastic syndrome (MDS) comprises heterogeneous group of clonal hematopoietic stem cell diseases with variable clinical course and risk of evolution to acute myeloid leukemia. Immune phenotypic changes of monocyte lineage are often seen in MDS. Aims: To evaluate functionality of monocytes with abnormal CD64 expression isolated from bone marrow of MDS patients. Methods: All samples were obtained after patients or controls signed an informed consent approved under CAEE number 33739214.0.0000.0071. All patient samples (15) and controls (15) were observed for presence of monocytes in CD64 expression and for monocytes subsets percentage using flow cytometry. Monocytes with abnormal CD64 expression were isolated using anti-CD56/CD14 magnetic beads. The isolated cells were cultured with GM-CSF and IL-4 in order to verify their ability of differentiation into dendritic cells. Monocytes phagocytic potential was also evaluated using phagocytosis assay with fluorescent E. coli bioparticles. Results: All patient samples presented phenotypic anomalies, and significant decrease in intermediate monocyte subpopulation compared to controls (p=0.0082). Dendritic cells derived from CD64 monocytes showed important morphological alterations in size and decreased/absence of dendrites formation. We also observed immunophenotypic changes in expression of molecules such as decreased HLA-DR and CD209 compared to controls (p=0.0035; p=0.049) respectively. Moreover, there was a significant decrease in phagocytic capacity in monocytes from MDS patients (p=0.001). Conclusion: Dendritic cells derived from MDS patients with or without aberrant expression of CD64 showed morphological and immunophenotypic alterations. Monocytes also displayed reduced phagocytic capacity. These changes are important and should be investigated in the context of disease development.

Investigating the Role of Tetraspanin 5 in Tumour Associated/Macrophages

M. Almoona1, P. Monk2, J. Saint Pol3, E. Rubinstein,4 M. Muthana

1UNIVERSITY OF SHEFFIELD, Sheffield, United Kingdom, 2Inserm/Universite Paris-Sud, Paris, France.

Rationale: Tumour-associated macrophages (TAMs) actively promote all aspects of tumour initiation, growth, and metastasis. Following exposure to tumour-derived factors we see a significant increase in TSAPNs. We hypothesise that TSAPN plays a role in supporting the pro-tumour activities of TAMs

Methodology: qPCR was used to determine the expression of TSAPN in human monocytes, monocyte derived macrophages (MDMs) and MDMs cultured in tumour-conditioned medium. Expression of TSAPN was also confirmed at the protein level. Accell siRNA knockdown was performed to determine the role of TSAPN in MDM phenotypic and function. NextSeq500 was performed to identify how TSAPN influences gene expression in BMDMs prepared from C57Bl/6 wild-type (WT) and TSAPN knockout (KO) mice. Findings: TSAPN was differentially expressed by monocytes and MDMs. Following differentiation from monocytes to MDMs, the expression of TSAPN was significantly reduced, however culturing MDMs from CD64 monocytes in tumour-conditioned medium significantly increased the expression of TSAPN. This expression was also evident in TAMs located in patient derived breast cancer tissue (n=20). Interestingly, this accounted for 44% of TAMs. Knockdown of TSAPN enhanced MDM migration and cell clustering and analysis of NextSeq500 (n=3) revealed significant changes in genes involved in cell adhesion (Fni, Cdh11, Lamb1, Cyni, Postn and Wisp2) and immune regulation (PTGS2, GPX8 and Crip2) in BMDMs from TSAPN mice compared to WT mice.

Conclusion: TSAPN is expressed by TAMs and this may regulate important macrophage properties. Future studies will focus on the role of TSAPN in preclinical mammary model in C57Bl/6 WT and TSAPN KO mice.

Cellular Mechanisms Controlling Surfacing of AICL Glycoproteins, Cognate Ligands of the Activating NK Receptor Nkp80

S. Neuss,1 Y. Barte2, C. Born,2 S. Weil,1 J. Koch,3 C. Behrends,2 M. Hoffmeister,4 A. Steinbre1

1Institute of Molecular Medicine, Goethe University Frankfurt, Frankfurt am Main, Germany, 2Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt am Main, Germany, 3Institute of Biochemistry II, Goethe University Frankfurt, Frankfurt am Main, Germany.

AICL glycoproteins are cognate activation-induced ligands of the C-type lectin-like receptor Nkp80 which is expressed on virtually all mature human NK cells. Nkp80-AICL interaction stimulates NK cell effector functions such as cytotoxicity and cytokine secretion. Notably, AICL and NKp80 are encoded by adjacent genes in the natural killer gene complex (NKC). AICL glycoproteins are cognate activation-induced ligands of the C-type lectin-like receptor NKp80 which is expressed on virtually all mature human NK cells. Nkp80-AICL interaction stimulates NK cell effector functions such as cytotoxicity and cytokine secretion. Notably, AICL and NKp80 are encoded by adjacent genes in the natural killer gene complex (NKC).

Background: AICL glycoproteins are cognate activation-induced ligands of the C-type lectin-like receptor NKp80 which is expressed on virtually all mature human NK cells. Nkp80-AICL interaction stimulates NK cell effector functions such as cytotoxicity and cytokine secretion. Notably, AICL and NKp80 are encoded by adjacent genes in the natural killer gene complex (NKC). AICL glycoproteins are cognate activation-induced ligands of the C-type lectin-like receptor NKp80 which is expressed on virtually all mature human NK cells. Nkp80-AICL interaction stimulates NK cell effector functions such as cytotoxicity and cytokine secretion. Notably, AICL and NKp80 are encoded by adjacent genes in the natural killer gene complex (NKC).
P.A1.02.16 Impact of obesity on the function and replenishment of hepatic macrophages in acute liver injury-mediated liver regeneration

G. Panota1, A. Assuma1, F. Gondor1, C. Kürts1, I. Föster1, H. Kastenmüller1, Z. Abdulla1,2
1Institute of Experimental Immunology, Bonn, Germany, 2Molecular Immunology and Cell Biology, Life and Medical Sciences Institute, Bonn, Germany.

Obesity often leads to chronic inflammatory liver diseases such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Increasing number of obese patients show a significant risk factor for drug-induced liver injuries and poor liver regeneration after resection of liver mass. Macrophages are key players, supporting tissue regeneration after injury. In this study, the composition of myeloid cells in livers of obese mice, was investigated with flow cytometry. Sorted liver macrophages from mice on high fat diet, were cultured with apoptotic cells or bacteria to determine their phagocytic capacity in vitro. Furthermore, acute liver injury was induced with carbon tetrachloride (CCL4) and serum levels of liver enzyme ALT were measured. Liver macrophages 30 days post injury, were stained with liver resident and bone marrow derived macrophage markers for flow cytometry and immunofluorescence. The composition of myeloid cells in livers of mice on high fat diet, have reduced Kuffer cell number and increased infiltration of neutrophils, inflammatory macrophages and monocytes. In addition, hepatic macrophage show impaired phagocytosis of both dead cells as well as bacteria in vitro. Furthermore, in the liver of mice on high fat diet, hepatic macrophages were unable to acquire a Kuffer cell phenotype following acute liver injury. Identifying the cellular and molecular mechanisms by which obesity might affect the function and phenotype of macrophages in liver injury is potentially open avenues to novel therapeutic concepts.

P.A1.02.17 Differential cellular programs of conditionally immortalized yolk sac and bone marrow macrophages

C. Schulz1, S. Stensch1, S. Ehlig1, A. Zehrer1, B. Wolz1, H. Häcker1, E. Hammer1,2
1Ludwig-Maximilians-University, Munich, Germany, 2Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, United States, 3Department of Functional Genomics, University of Greifswald, Greifswald, Germany.

Introduction: Yolk sac (YS) hematopoiesis gives rise to tissue macrophages in many organs. However, the cellular program of these macrophages is incompletely understood.

Methods/Results: We conditionally immortalized hematopoietic precursors of YS and bone marrow (BM) origin using estrogen-inducible HOX8, and differentiated them into mature macrophages. We than compared in detail their cellular identity and functions. Using proteome and gene expression analysis, we found that macrophages derived from BM progenitors developed a phenotype reminiscent of classically activated macrophages, whereas macrophages differentiated from YS progenitors displayed an increased abundance of anti-inflammatory proteins. Further, we identified differences in macrophage metabolism and functions. Conclusions: Our findings suggest that, when compared under standardized conditions, the distinct molecular programs are present in YS vs BM macrophages. This adds a novel perspective to the regulation of macrophage functions and could provide opportunities for their therapeutic Manipulation.

P.A1.02.18 Towards a better understanding of lung interstitial macrophages identity

J. Schyms1, D. Pinotti2, F. Burel3, T. Marichal3
1Laboratory of Cellular and Molecular Immunology, GIGA-Research, University of Liège, Liège, Belgium, 2Faculty of Veterinary Medicine, University of Liège, Liège, Belgium, 3Wallon Excellence in Life Sciences and Biotechnology (WELBIO), Wallonia, Belgium.

Lung interstitial macrophages (IM), constituting the non-alveolar lung macrophage compartment, have been shown to exhibit tolerogenic properties at steady-state by inhibiting the ability of dendritic cells to induce allergic type 2 responses against inhaled allergens. Recently, several reports have provided experimental evidence that IM represent a heterogeneous population in the steady-state lung, and encompass at least two subpopulations, each of them likely carrying their own identity, e.g., phenotype, localization, differentiation program, and function. In order to assess the heterogeneity of IM in an unbiased way, droplet-based single-cell RNA sequencing experiments were performed on the IM pool, revealing three subpopulations of IM at the steady-state. Differentially expressed genes among the subpopulations allowed us to find specific surface markers that identify these phenotypically and functionally distinct subpopulations by flow cytometry, a crucial step to assess the identity of IM, and how such identity is imprinted by the local environment to fulfill the functional needs of the lung mucosa.

P.A1.02.19 IFN-γ-induced STAT3 activation as an alternative pathway in acute myeloid leukemia

G. Tundal1, D. Yayan-Emsa, G. Esendagli
1Hacettepe University Cancer Institute, Department of Basic Oncology, Ankara, Turkey.

Introduction: Even though IFN-γ is implicated in anti-tumor immunity, immunoregulatory pathways can simultaneously induce upon exposure to this cytokine. In addition to signal transducer and activator of transcription 1 (STAT1), which is a main pathway that leads to cytostatic and immune-provoking effects of IFN-γ, STAT3 can also be activated. STAT3 promotes cancer cell growth, survival and immunosuppression. Here, we investigated the influence of IFN-γ exposure on STAT3 induction in acute myeloid leukemia (AML) cells.

Methods: AML cell lines (THP-1, U937, HL-60) were treated with all-trans retinoic acid (ATRA) or 1α,25-dihydroxyvitamin D3 (D3) to modulate their differentiation status. Maturation of AML cells were assessed by cytometry and flow-cytometric immunophenotyping. Bone marrow samples from AML or myelodysplastic syndrome patients and CD11b+ myeloid cells from healthy donors were also studied. Cells were stimulated with recombinant IFN-γ for different time periods. Phospho-STAT3 (pSTAT3), total-STAT3 (tSTAT3), pSTAT1 and tSTAT1 levels were assayed by Western-Blot.

Results: STAT3 was efficiently induced with transient IFN-γ stimulation and pSTAT3 levels were compatible to that of pSTAT1. Hence, all samples studied were responsive to IFN-γ. This effect was augmented when AML cells previously underwent ATRA or D3 maturation conditions. STAT3 pathway was transiently activated in healthy myeloid cells but long-term (48h) exposure to IFN-γ had a positive feedback effect on pSTAT3 and tSTAT3 levels in AML cells.

Conclusion: In contrast to its anti-tumor actions, IFN-γ can effectively stimulate STAT3 in AML conditions, supporting immunosuppressive characters and pro-survival activities. Targeting STAT3 in AML may complement immunotherapies which are related to IFN-γ-mediated immunity.

P.A1.02.20 Monocytes bearing G551-D-CFTR mutation present a high RANK/MCSF co-expression: first evidence of a facilitated osteoclastogenesis in cystic fibrosis patients?

M. Journiaux1, D. Abdallah1, C. Guillaume1, N. Ronay1, Y. McCarthy1, E. Flanagny1, B. J. Plant1, I. Jacquot1, F. Velard3
1EA 4691 BIOS, Reims, France, 2University College Cork National University of Ireland, Cork Cystic Fibrosis Center, Cork, Ireland, 3University College Cork, Dept. of Respiratory Medicine, Cork, Ireland.

Bone fragility and low bone mineral density occur in cognitively normal children and young adults with cystic fibrosis (CF) disease. Due to the presence of CFTR in monocytes, we hypothesized that it may impact monocyte differentiation and activation in osteoclasts. Osteoclast precursors fuse and differentiate to form bone-resorbing multinuclear osteoclasts upon stimulation by the receptor activator of NF-kappaB ligand (RANKL) and the macrophage-colony stimulating factor (M-CSF). Despite the presence of CFTR in monocytes, we hypothesized that it may impact monocyte differentiation and activation in osteoclasts. Osteoclast precursors fuse and differentiate to form bone-resorbing multinuclear osteoclasts upon stimulation by the receptor activator of NF-kappaB ligand (RANKL) and the macrophage-colony stimulating factor (M-CSF). This hypothesis was supported by the presence of CFTR in monocytes. We then investigated the expression level of M-CSF and RANK and RANK receptors on blood monocytes from G551-D CF patients by flow cytometry, prior to and at nine and twelve months after receiving CFTR potentiator ivacafer. Compared to healthy controls, our first set of data demonstrates higher level of a double M-CSF-RANK+RANK receptor on monocyte of G551-D CF patients, which was reduced in vivo ivacafer treatment. Moreover, we examined ex vivo differentiation and activation of healthy monocytes into osteoclasts for a 21-days period with/or without the addition of 1nH-172 drug, an inhibitor of CFTR chloride channel activity. Interestingly, multinuclear osteoclasts from inH172-treated healthy monocytes were more adherent, and were prone to generate large pits and trenches of dentin resorption. In addition, multinuclear osteoclasts derived inH172-treated healthy monocytes released reduced level of bioactive lipid mediator sphingosine 1-phosphate (S1P), a key mediator in the directed migration of osteoblast/osteoclast precursors attached to the bone surface. Altogether, these data highlight the critical regulatory role of CFTR in M-CSF and RANK receptors expression in monocytes, and suggest CF bone disease as a new, cell-type monocyte dysfunction disease. Vaincre la Mucoviscidose and Vertex Inc. provided funding supports.
A hallmark of Alzheimer’s disease pathology is neurofibrillary tangles comprising hyper-phosphorylated tau. Microglia are resident myeloid cells of the CNS that are implicated in neuro-inflammatory and neurodegenerative disorders. To investigate the reciprocal relationship between microglia and tau pathology we first characterized the microglial response in a model of progressive tau accumulation (hTau mice). We did not detect changes in microglia surface receptor expression on proliferation, cytokine production, morphology or transcriptional profile in aged hTau mice indicating a lack of pathogenic microglia responses to tau aggregation. To assess the direct impact of microglia on tau pathology and associated neurodegenerative deficits we developed a protocol for long term microglial depletion in CX3CR1CreERT2R26R/2 mice and crossed them with hTau mice. We then depleted microglia for 3 months which resulted in exacerbation of spatial memory function. These results indicate that microglia have a neuroprotective role during Alzheimer’s related tau pathology.

Newborn babies have high number of immature monocytes expressed CD116 in peripheral blood than adults

Y. Koker1, S. Özsoy, B. Bingöl, S. Kütkü, C. Karaca; Erciyes medical school, Kayseri, Turkey.

Monocytes are able to differentiate to dendritic cells (DCs) under inflammatory situations. Different monocyte subsets show distinct inflammatory cytokine profiles and differentiation potential under steady-state and inflammatory situations. The major subset of monocytes consists of CD14-high CD16-negative (CD14++CD16−). Committcd dendritic cell originated from immature monocytes. In humans (hDCs) that develops from committed DC progenitors (hDCPs) in the BM. We have measured the number of immature monocytes (pre-DCs) with CD34+ and CD34+. CD16+ expression by flow cytometry with acquisition of a million cells from peripheral blood in 10 newborn and 10 adults. To determine the physiological distribution of pre-DCs in humans, we examined, peripheral blood, of newborn and adult for small numbers of pre-DCs travel through the blood and replace cDCs in the peripheral organs, maintaining homeostasis of the highly dynamic cDC pool. Monocyte-derived circulating short-lived pre-DCs are high in newborn (mean:57 cells/million cells) than adults (mean:7 cells/million cells). We assume that any organ includes epithelial cells, endothelial cells, fibroblasts, stromal cells, and hematopoietic cells are a source of GM-CSF secretion. Circulations of CD116+ short-lived pre-DCs undergo maturation when going through the vascular environment with high GM-CSF secretion to microenvironment.

Suu72 phosphatase regulates tissue-resident macrophage function

E. Park1, S. Lee1, C. Lee1 2; 1Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon 16419, Korea, Republic of; 2Department of Health Sciences and Technology, SAHST, Sungkyunkwan University, Seoul 06352, Korea, Republic of.

Dynamic alterations of phosphorylation state of many cellular signaling-mediated proteins regulate their molecular and cellular fates. Suu72 is dual protein phosphatase that can act upon tyrosine or serine/threonine residues and transcription/RNA processing factor. Suu72 has been characterized as an RNA polymerase II carboxy-terminal domain phosphatase that specifically catalyses serine-5-phosphorylation. Recently, we reported that Suu72 functions as a cohesin-binding phosphatase and interacts with Aurora B kinase for regulation of duplicated sister chromatid separation, and that the deletion of hepatocyte specific Suu72 led to the development of a high incidence of fatty liver diseases. Suu72 is known to be expressed in a tissue-specific manner, and we found that Suu72 expressed in adipose tissue, especially strongly in brown adipose tissue. In this aspect, we generated conditional knock out mice which Suu72 is deleted specifically in adipose tissue and found that the deficiency of Suu72 leads to BAT dysfunction compared to wild type mice. Interestingly, we observed not only dramatically reduction of macrophage population but also defective M2 macrophage generation in Suu72-deficient BAT. Thus, we further generated myeloid cell specific Suu72 knockout mouse model. This study will include the physiological relevance of Suu72 loss-of-function in tissue-resident macrophage.

Immune development and aging from the cradle to the grave - Part 1

Differential Recovery Of Intrathymic Microenvironments That Follows Thymus Damage Results In Qualitative Changes In T-cell Reconstitution

A. Alawam, A. J. White, W. E. Jenkinson, G. Anderson; University of Birmingham, Birmingham, United Kingdom.

Following ablative therapies used for cancer treatment, damage to the thymus disrupts its ability to support T-cell reconstitution. This results in delayed T-cell reconstitution and a period of immunodeficiency that leaves patients susceptible to potentially fatal infections. Thus, examining mechanisms that control thymus regeneration, and identifying new approaches to boost thymus recovery, is important in devising new therapeutic strategies to improve immune reconstitution.

We have used sub-lethal irradiation (SLI) in a mouse model of thymic injury, and performed systematic examination of the recovery of both thymocytes and the thymic microenvironment. Following SLI, we find the generation of CD4-CD8- thymocytes and their CD4+ and CD8+ progeny occurs in two distinct waves. Analysis of early thymic progenitors indicates that while an initial and transient wave of thymus recovery occurs via a radioreistant intrathymic progenitor, a second sustained wave occurs via thymus entry of bone marrow progenitors. Surprisingly, concurrent analysis of the thymic microenvironment indicates cortical thymic epithelial cell numbers remain constant, suggesting they are radioreistant and available to support thymocyte development. In contrast, medullary thymic epithelial cells and dendritic cells are depleted following damage. Consistent with this, recovery of medulla-dependent Foxp3 regulatory T-cells occurs after the generation of conventional CD4+ thymocytes.

In summary, our findings suggest that following damage, distinct thymic areas show differential recovery kinetics that impact upon the quality of new T-cell production. Ongoing studies are examining whether known regulators of thymus recovery, including KGF and LTβR stimulation, can restore medullary microenvironments to ensure balanced recovery of thymocytes and Treg.

Immune development and aging from the cradle to the grave - Part 2

Effect of IL-15 cytokine on the adhesion and migration properties of CD4+CD28null T-lymphocytes in rheumatoid arthritis patients

M. A. Moro García1, L. García Jartín1, E. Bueno García1; 1Deparment of Clinical Neuroscience, Applied Immunology and Immunotherapy, Karolinska Institutet, Stockholm, Sweden, 2Center for Molecular Medicine, Karolinska Hospital at Solna, Stockholm, Sweden, 3Department of Women's Health and Children's Health, Karolinska Institutet, Stockholm, Sweden.

Centro de Investigaciones Biomédicas (CINBIO), Universidade de Vigo, Vigo, Spain.

Effect of IL-15 cytokine on the adhesion and migration properties of CD4+CD28null T-lymphocytes in rheumatoid arthritis (RA). The main objective of this work was to study the adhesion and migration capacity of these cells and the effect of the cytokine IL-15 on these properties. The experiments were performed with peripheral blood samples from patients with RA, where the CD4+ T-lymphocytes were isolated. To study the adhesion ability of CD4+CD28null cells CD11a, CD49d, CD44,CCR5 and CX3CR1 molecules were studied by flow cytometry. The basal level of all these molecules was higher in CD4+CD28null T-lymphocytes. Moreover, IL-15 induced a significant increase in CD11a and CD44 in CD4+CD28null T-cells. Cell migration was studied in CD4+ T-lymphocytes isolated and cultured in "transwell". Migrated cells were significantly higher in the wells with IL-15 and the majority of these cells were CD4+CD28null. This effect was IL-15 dose and time dependent. We also studied the activation and activity of Rho A, Rac1 and Cdc42, proteins involved in cell migration. Both the basal levels of Rho A and the activated RhoA, Rac1 and Cdc42 were clearly higher in CD4+CD28null T-cells. In conclusion, CD4+CD28null T-cells exhibited very different migratory and adhesion properties compared to CD4+CD28+ T-cells in RA. This could be interesting when designing different therapeutic targets to try and prevent the migration and accumulation of these cells in locations where they could exert a pathogenic function and thus, slow down the progression of the disease.
Acute and subacute trafficking within and outside of early life lymph nodes

F. Auerst,1 A. Rochat,1 2, P. Fontanot,1 2, C. Tougue,2 3, P. Lambert,2 3, C. Siegrist1 2,3
1Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland, 2World Health Organization Collaborating Center for Vaccine Immunology, Faculty of Medicine, Geneva, Switzerland, 3Center for Vaccinology, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland.

A key determinant of B cell responses is the delivery of antigens to B cells and follicular dendritic cells. Small soluble antigens gain access to B cell follicles through the conduit system, whereas larger antigens are captured by the subcapsular (SCS) macrophages lining the sinus of the lymph node (LN) and transferred to follicular B cells. Neonatal immunity is known to elicit poor and delayed germinal center (GC) B cell responses, suggesting suboptimal B cell targeting and activation. Given their role in antigen capture and delivery, we studied the postnatal development of SCS macrophages and show that they develop very slowly after birth, appearing in LNs only at 3 weeks of age. To investigate the consequences of their absence in neonates, we compared the fate of fluorescent small and large antigens by injecting OVA-FITC or PE, respectively, adjuvanted to the AS03 oil-in-water adjuvant. Surprisingly, we observed AS03 Ag- cells in contratral non-draining LNs of 1-week-old and 3-week-old mice 24 hours post-inoculation, independently of the size of the antigens.

This resulted in local activation and proliferation of CD4+ T cells and development of GCs. This process was oil-in-water adjuvant-dependent and likely resulted from the expression of monocyte chemo-attractants in distant LNs. Our data suggest lymph node impaired barrier and systemic diffusion of vaccine formulations containing oil-in-water adjuvants. Overall, ontogeny exerts a profound influence on antigen/adjuvant retention and diffusion, which should be taken into account when designing vaccine formulations considered for use in early life.

Acetylation of PLZF regulates the lineage specification of INKt7 cells

C. Joseph,1 J. Klabi2 3, M. Dolead4 5, V. Pariente5, M. Pio5, B. Lucas6, C. Chamoinne4, A. Toubert7, F. Gui1, k. benlagha8

The transcription factor PLZF (promyelocytic leukemia zinc finger) encoded by the BTB domain containing 16 (Zbtb16) gene is expressed during the development of invariant natural killer T (iNKt7) cells to direct the acquisition of their effector program. In this study we addressed the role of PLZF acetylation in INKt7 cells by analyzing the development these cells in mice expressing a constitutive acetylated form of PLZF (PLZFom mice). We found that the constitutive activation of PLZF repressed the development of all iNKt7 cell subsets (NK1.1, NK1.2, and NK1.17). This developmental block is intrinsic to iNKt7 cells as assessed by reconstitution experiments. In these mice iNKt7 cells are blocked at the CD44+ stage, do not upregulate IL-17Rb, and produce mostly IL-4 and IL-13, indicating that the block occurs at an early precursor stage. Finally, we found in normal mice that the acetylated EP300, known to induce PLZF acetylation in vivo, is expressed at the early CD44+ stage of development, indicating that a similar activation could take place during normal development. In addition, we found that the deacetylase HDAC3 and SIRT1, also PLZF partners, are expressed at the CD44+ stage, indicating that PLZF suppression could be alleviated at the subsequent developmental stage. Overall, our study reveals a non-expected suppressive rather than activating role of PLZF while promoting iNKt7 development and proposes a model where a tight control of its acetylation/deacetylation during developmental stages regulates lineage specification of this population.

The adjuvant LT-K63 overcomes neoplastic lesions in germinal center induction by circumventing the induction of suppressive regulatory cells

S. P. Bjarnason,1 2, A. Aradottir Pind3, 4, G. Magnusdottir3, 4, G. dei Giudici5, I. Jonsdottir3 4, A. A. Aradottir Pind1, 2
1Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavik, Iceland, 2Reykjavik, Iceland, 3Department of Immunology, Landspitali, the National University Hospital of Iceland, Reykjavik, Iceland, 4GIK Vaccines, Siena, Italy.

Introduction: High susceptibility to infectious diseases and low vaccine responses characterizes the neonatal and infant period due to immature immune system. Formulating antigens with adjuvants may enhance vaccine immunogenicity and efficacy. T follicular helper (Tfh) cells play an important role in germinal center (GC) reaction, production of antibody secreting cells (AbSCs) and memory B cells, whereas follicular T regulatory cells (Tfr) can suppress Thf and GC B cells numbers. We evaluated the effects of the adjuvant LT-K63 on Tfh and regulatory cells in relation to its enhanced neonatal vaccine-induced humoral response. Materials and Methods: The frequency of Tfh, Tfr regulatory T cells (Tregs), IL-10-secreting B cells (B10), GC B cells, plasmablasts and plasma cells, was assessed in 7-28 days old and adult mice, and 4, 8, 14 and 21 days after neonatal parenteral immunization with pneumococcal conjugate (Pnc1-TT) w/o LT-K63. Vaccine-specific AbSCs and Abs were also measured. Results: Maturation of Tfh, Tfr cells, Tregs and B cells was age-dependent, in neonates a larger fraction of CD4+ T cells and B cells differentiated into IL-10-secreting Tregs or B10 and B10-phenotype differed. Upon immunization LT- K63 enhanced neonatal induction of Tfh compared to vaccine alone and increased the ratio of Tfh/Tfr, but decreased Treg and B10. Accordingly, LT-K63 enhanced the induction of GC B cells, plasmablasts, plasma cells, vaccine-specific AbSC and Abs. Conclusion: The adjuvant LT-K63 contributes to enhanced Tfh differentiation and induction of GC B cells by circumventing the induction of suppressive regulatory cells, contributing to enhanced and persistent vaccine responses.

Telomere length on individual chromosome arms in patients with immunopathology and healthy donors

M. S. Barkovskaya, E. A. Blinova, J. V. Konyahina, A. E. Sizikov, D. V. Demina, V. A. Kazlov; Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

Telomere length is an important factor characterizing immune system, its decreasing indicates a premature aging in immune-mediated diseases. The distribution of telomere repeats on different chromosomes has an individual telomere profile in humans. The purpose of this study was to estimate the telomere lengths in the individual chromosome arms patients with rheumatoid arthritis (RA), [n=6, the median age 51.5 (50-54)] and bronchial asthma (BA), [n=6, the median age 43.5 (37-57)] in comparison with healthy donors. Two groups of donors were selected strictly consisting of 6 healthy donors. Written informed consents were obtained from persons enrolled in the study. RA and BA were diagnosed by clinicians according to ACR/EULAR-2010 and GINA-2017. Metaphase spreads obtained from PBMCs were used. For measurement of the telomere length Q-FISH with (Cth) probe was applied. The new MeTelLen software was developed to estimate the telomere repeats frequency http://www.bionet.nsc.ru/en/application/application-development/development-of-a-computer/metelen.html in metaphase images. When comparing the telomere length, it was found, that telomeres on p-arms of chromosome 15 in RA and 9 in BA are significantly shorter than in corresponding group of donors (p<0.05, Mann-Whitney U-test). Also the common telomere profiles obtained from groups of patients and donors were evaluated. Analysis revealed a number of identical and distinctive features in distribution of significantly short (9q, 15q, 17p, 22q) and long (3p, Xp) telomeres (Wilcoxon-signed-runk test). This may be consequence of a proliferative stress in the immunopathology or instead a congenital feature, that accelerates immunosenescence and play role in pathogenesis of studied diseases.
POSTER PRESENTATIONS

P.A2.01.08 Immunomodulatory activity of Lactobacillus isolated from human milk and baby stools
M. C. de Almagro, G. Cifuentes, M. García, J. A. Moreno, J. Jiménez, M. Rodríguez-Palomo; Laboratorios Ordesa, Sant Boi, Spain.

During the first months of life, babies have an immature immune system. Breastmilk contains different immunomodulatory compounds that help the infant fight infections and favors a proper maturation of the immune system. Among these breastmilk components bacteria can be found. Probiotics are bacteria regarded as “good bacteria” due to their health benefits when used correctly. To identify new functional probiotics, a proprietary library of Lactobacillus from human milk and stools from babies exclusively breastfed were screened for immunomodulatory activities. First, probiotics safety was assessed by measuring the cytotoxicity produced by the bacterial lystate as well as the bacterial supernatant in TH29 and THP1 cells. Lactobacillus ability to modify the levels of 11 cytokines was measured by Lumixem and ELISA in basal conditions and upon LPS or TNFa treatment, obtaining several candidates with antiinflammatory and tolerogenic characteristics (IL-10 secretion and TNFa decrease upon challenge). Among those candidates, two strains with good growth properties up to the pilot scale, were selected. Therefore, two Lactobacillus strains isolated from baby stools with immunomodulatory activity which could potentially be used in baby formula have been found. Support: this project is supported by Centro para el Desarrollo Tecnológico Industrial (CDTI).

P.A2.01.09 VDJ-recombination shapes the clone-size distribution of naive T-cells
P. C. de Greep, T. Oakes, B. Gerritsen, J. Heather, R. Hemsen, B. Chain, R. I. de Boer; 1Utrecht University, Utrecht, Netherlands; 2University College London, London, United Kingdom; 3Iboly School of Medicine, New Haven, United States.

The clonal distribution of the human naive T cell receptor (TCR) repertoire is unknown, and measuring it is not feasible because of the vast repertoire diversity. We report a strong relation between the frequency of TCR alpha- and beta-chains of naive T-cells in blood samples from adult volunteers and the probability of these sequences to be generated by V(D)J-recombination in the thymus. This strong relation is unexpected because in adults, only a small fraction of naive T-cells is produced by the thymus, while the vast majority are a result of peripheral division. To examine if VDJ-recombination probabilities can indeed explain the frequency differences between TCRs in the naive compartment, we developed simple mathematical models describing naive T-cell dynamics, and compared their predictions with sequencing data. We establish the presence of a small fraction, but a large number, of TCR-sequences that are abundant and frequently generated by the thymus (i.e. the alpha- or beta-chains have a high VDJ-recombination probability). These results demonstrate an unexpectedly large role for VDJ-recombination probabilities in shaping the naive clone-size distribution, casting doubt on the role that cognate signals play in determining clonal-sizes of naive T-cell repertoires.

P.A2.01.10 Young serum and its-derived extracellular vesicles potentiates rejuvenated aged T-cell immunity & immune tolerance (Supported by NIH RO1AI121147 to D-M. S.)
S. Dong-Ming, J. Oh, W. Wang; University of North Texas Health Science Center, Fort Worth, United States.

Aging results in thymic involution, with decreased output of naive T-cells resulting in accumulation of exhausted CD28γδ T-cells, involved in immunosenescence; and increased output of self-reactive T-cells, related to development of self-reactivity-resulted chronic inflammation. Both conditions establish a risk for increased morbidity and mortality in the elderly. Therefore, it is of great importance to develop rejuvenating strategies able to attenuate these. A promising “rejuvenation factor” present in young blood has been found to be able to make aged neuron “younger”. However, it is uncertain whether and how young serum-derived factors work on aged immune system. Herein, we tested rejuvenation in aged human (>70YO) peripheral blood mononuclear cells (PBMCs) with young human serum in humanized mouse model, and in the naturally-aged mouse (>16MO) atrophied thymus with young mouse serum-extracted extracellular vesicles(EVs)/exosomes, in which full of epigenetic regulators are encapsulated. We determined young human serum, but not PBS, was able reduce CD8αα cells in CD4 and CD8 subsets, with reduction of global methylation, and increased cell numbers and activation of Bim cells. We also found young murine serum-derived EVs, but not non-EV supernatant, were able to attenuate inflammation in both the periphery and central nerve system of old mice, attributed to partially reversed thymic involution and improved function in thymocyte negative selection. Our results in rejuvenation of T-cells on both peripherial immunosenescence and central immune tolerance serve as an impetus to encourage us to determine the underlying mechanism, which is hypothesized due to young serum-provided epigenetic intervention.

P.A2.01.11 Distinct pattern of differentiation and responsiveness of chicken CDBααγδ γδ T cells
M. Gu, K. Yu, F. Kye, B. Park, T. Park, S. Han, C. Yum; Seoul National University, Seoul, Korea, Republic of

The aim of the present study was to investigate phenotypically distinct subset of γδT cells in the periphery and, more importantly, their function. In chickens, CD8 expression subdivides peripheral γδT cells into 3 subsets (CD8+ αα, CD8- ααβ, and CD8+ αβ) γδT cells). Interestingly, unlike other subsets, CD8ααγδ cells were absent in the thymus while they are present in all other organs examined. It is important to note that CD8ααγδ cells did not convert into other subsets when stimulated with PMA/IL-2 whilst other subsets were able to become CD8ααγδ. Furthermore, CD8ααγδ cells in the periphery exhibited high expression of CD25, CD28 and CD44 compared to other subsets. These results indicated that chicken CD8ααγδ cells are most likely the formerγ, allogeneically differentiated subset. To further investigate the response to innate and TCR stimuli, we prepared supernatant from total splenocytes treated with LPS (LPS supernatant), which contains major inflammatory cytokines (IL-1β, IL-6 and IL-23), γδT cells were treated with the LPS supernatant and/or acdc32/33 monoclonal antibodies (for TCR stimulation). We found that LPS-induced mediators induced proliferation of CD8ααγδ γδ T cells which was mostly CD8 negative, while TCR stimulation induced proliferation of CD8+ ααβ γδ T cells. These results suggested that proliferation of CD8ααγδ γδ T cells with a distinct CD5 expression pattern displayed distinct response to innate and TCR stimuli, which may help for investigating and understanding their functional role in infection and inflammation.

P.A2.01.12 Lineage specification of promyelocytic zinc finger-expressing innate iNKT cells is controlled by strength of TCR signaling
C. Joseph,1, J. Kilb1,2, L. Amabile2, J. Combati1,2, A. Cascoferro2, M. Delord2, V. Parietti1,2, C. Lenzo2, S. Lotour2, B. Lucas2,3, C. Viet2,3, A. Touber2,3, K. Benlagha1,2; 1Inserm, paris, France; 2INSERM, UMR1160, Institut Universitaire d'Hématologie, Paris, France; 3Université Paris Diderot, Sorbonne Paris Cité, Paris, France; 4Institut Pasteur, paris, France; 5Unité de Pathogénomie Mycobactérienne Intégrée, Institut Pasteur, 75248, France, Paris, France; 6Université Paris Diderot, Sorbonne, Paris, France; 7Plateforme de Bio-informatique et Bio Statistique, Institut Pasteur Université Paris Diderot, Sorbonne Paris Cité, Paris, France; 8Département d'Expérimentation Animale, Institut d'Hématologie Paris, France; Université Paris Diderot, Sorbonne Paris Cité, Paris, France; 9Institut Curie, Centre National de la Recherche Scientifique UMR8004, INSERM U1106, Université Paris Descartes, Paris, France; 10ICR, International Center for Infectology Research, Université de Lyon, Lyon, France; 11INSERM, U1111, Lyon, France; CNRS, UMR5308, Lyon, France.

Natural Innvariant Killer T (iNKT) cells are innate-like T-cells, the development of which depends on the transcription factor PLZF. We aimed in this study to assess the contribution of the canonical TCR Va14-Ja18 alpha chain in driving iNKT1, 2, and 17 developmental programs. Analysis of iNKT cell development in conventional Va14-Ja18 transgenic (Tg) mice, where the Va14-Ja18 transgene is expressed as early as the CD4+CD8 γδ T-cells, showed that the Va14-Ja18 chain does not favor the entry of iNKT cells into a specific developmental program. However, we found that iNKT cell specification is affected in CD4+Va14-Ja18 Tg mice, where the transgene is controlled by a CD4 promoter retaining its expression at the CD4+CD8 γδ positive stage. In these mice, we observed an increase in the T-cell BCRIγδ subset that we found to represent early iNKT cell precursors rather than NK1.1 cells. These cells have a block in the transition from the developmental CD44γδ stage 1 to CD44αβ stage 2 and a defect in acquiring IL-17RA at the CD44αβ stage 1, revealing an earlier developmental block. Analysis performed at the transcription and protein levels showed a reduced expression of Egr-2 and its target gene PLZF in CD4+Va14-Ja18 Tg mice. Moreover, we observed that these cells perceived a weaker TCR signal that is likely at the origin of their altered downstream TCR signaling and subsequent developmental defect. Overall, our study highlights TCR signal strength, and not Va14-Ja18 chain, as an important regulator of iNKT cell lineage specification.
...for CD3+ and CD3+CD4+ gates, not for CD3+CD8+. We suppose that ‘pre-mature ageing’ of some lph’s populations can be an unspecific event in chronical immune diseases (MABs). The CD45RA/CD45RO expression was examined in the gates of T-cells (CD3+), T-helpers (CD4+) and T-suppressors (CD8+) activity. The CD45RA and CD45RO membrane expression on peripheral blood lphs was determined with four-color flow cytometry (BD FacsCalibur, Cell-Quest Software, BD Biosciences MultiTest CD45RA/CD45RO/CD3/CD4 & CD45RA/CD45RO/CD3/CD8 MABs). The CD45RA/CD45RO expression in 25 children with autoimmune hepatitis (AIH) and 25 with chronical hepatitis C (HC), groups were equivalent with ages (7-15 y.o.), genders, and disease duration of illness. Accumulation of CD45RA+ and CD45RO+ lphs was not different between the groups, but CD45RO+ expression of CD3+ cells was significantly lower in AIH patients compared to HC patients (p<0.05) and was strongly linked to increased viral load (p<0.001). The CD45RA/CD45RO expression on CD4+ and CD8+ lphs did not vary significantly between the groups. We conclude that ‘pre-mature ageing’ of CD45RA+ lphs may be a common feature of autoimmune and chronical viral hepatitis; however, more studies are needed to confirm this finding and to understand the biological significance of this phenomenon.
Nuclear receptor corepressor 1 (NCOR1) is a transcriptional regulator bridging repressive chromatin modifying enzymes with transcription factors. NCOR1 regulates many biological processes, however its cellular functions are not well known. Here we show that CD8+ TCRα-β+ naive T-cells deficient of NCOR1 (NCOR1-/-) resulted in a reduction of peripheral T cell numbers due to a decrease in single-positive (SP) Tocytes. In contrast, double-positive (DP) Tocyte numbers were not affected in the absence of NCOR1. The reduction in SP cells was due to diminished survival of NCOR1-null postselection TRAB+CD69+ and mature TRAB+CD69+ Tocytes. NCOR1-null Tocytes expressed elevated levels of the pro-apoptotic factor BIM and showed a higher fraction of cleaved caspase 3-positive cells upon TCR stimulation ex vivo. However, staphylococcal enterotoxin B (SEB)-mediated deletion of Vβ8+CD4SP thymocytes was not altered in the absence of NCOR1. Finally, transgenic expression of the pro-survival protein BCL2 restored the population of CD69+ Tocytes in NCOR1 cKO mice to a similar percentage as observed in WT mice. Together, these data identify NCOR1 as a crucial regulator of the survival of SP Tocytes and revealed that NCOR1 is essential for the proper generation of the peripheral T Tcell population.

We found a highly significant association between acid-inhibiting and subsequent anti-allergic drug prescriptions. These findings were more prominent in women (p<0.001 compared to males). Age and gender adjusted hazard ratios were selected in a region-based data set (n=1.27 million) using a population-based analysis, covering 8.2 million (approx. 97%) of the Austrian population. Consecutive prescriptions of gastric acid-inhibitors (PPI, H2-receptor antagonists, sucralfate, prostaglandin E2) followed by anti-allergics were analysed in a regional subgroup (Burgenland) controlling for age and gender.

The association between gastric pH modulation and allergic sensitization has been reported. To evaluate this suspected association, we aimed to assess the frequency of prescribed CHMPs as a key sensor of TCR signal thresholds that promote Tocyte selection. Notably, Tocyte-specific loss of CHMPs abolished T-cell development in a manner partly dependent on the ability of CHMPs to stabilize Bcl-2 proteins in TCR-signalized Tocytes. Absent CHMPs, positively selected Tocytes underwent apoptosis that was partially rescued by genetic deletion of the apoptosis activator Bim or by transgenic overexpression of Bcl-2. We found that not only was CHMPs posttranslationally stabilized by the deubiquitinase USP8, it also functioned as an adaptor to orchestrate normal client protein substrate ubiquitination in developing Tocytes. Collectively, our studies have uncovered an unexpected checkpoint during positive selection mediated by CHMPs and identify CHMPs as an essential component of the posttranslational machinery required for T cell development.

Human MAIT cell count forms a surrogate for general immune health

Human mucosal associated invariant T-cells (MAIT) form a subset of CD8 T-cells with recombined T-cell receptor (TCR) alpha chains containing the product of TRAV2-J with either TRAV1-J2-ALPHA. MAIT cells have a distinct gene expression profile with high expression of KLRB1 encoding CD161 and RORC encoding the transcription factor RORyt. Whilst MAIT cells are found from mice to humans, the determinants of their gene expression pattern in humans are poorly understood. Using TCR mapped from purified CD8 T-cell RNA-sequencing data (196 samples from 102 individuals) we define a proxy for MAIT count with which we deconvolute a MAIT specific gene signature that out-performs single-cell sequencing data from the same individuals. We replicate previous findings of a rapid decline in MAIT count with age and we further find that MAIT count correlates, however in a role in immune health including T-cell clonality and anti-viral and pro-inflammatory gene expression signatures in NK cells and monocytes, from the same individuals, respectively. Within MAIT cells we find 155 genes whose expression is associated with age, including AIRH, FTTPRB and GPR15. We also observe 20 sex associated genes within MAIT cells. Finally, we demonstrate a number of other TCR within CD8 T-cells are correlated with MAIT count, suggesting shared factors controlling MAIT cell count over life and other subsets.

Human MAIT cell count forms a surrogate for general immune health

Background: Anti-ulcer medications, such as proton pump inhibitors (PPI), are amongst the most frequently prescribed drugs in Europe. In recent years, however, evidence for an association between gastric pH modulation and allergic sensitization has been reported. To evaluate this suspected association, we aimed to assess the frequency of prescribed background allergy treatment following acid-inhibitors prescription in Austria. Methods: Data from health insurance claim records 2009-2013 were assessed in a population-based analysis, covering 8.2 million (approx. 97%) of the Austrian population. Consecutive prescriptions of gastric acid-inhibitors (PPI, H2-receptor antagonists, sucralfate, prostaglandin E2) followed by anti-allergics were analysed using data from Austria-wide. As control condition, consecutive prescriptions of other common medications (lipid-modifying and antihypertensive drugs) followed by anti-allergics were analysed in a separate analysis. Results: The specific hazard for the prescription of anti-allergic drugs was doubled in the overall population (1.96 [95% CI:1.95-1.97]) and triplicated in the regional data set (3.07 [95% CI:2.89- 3.27]), after prescription of gastric acid inhibiting drugs (p<0.001). These findings were more prominent in women (p<0.001 compared to males). Age and gender adjusted hazard ratio was 2.05 [95% CI:1.91-2.19], and elevated independent of preceding acid-inhibitor type. The risk increased age-dependent from 1.47 [95% CI:1.45-1.49] in <20 year olds, up to 2.05 [95% CI:1.91-2.19] in >60 year olds. Conclusion: Significant correlation between acid-inhibitors and subsequent anti-allergic drug prescriptions. These population-wide findings are in line with previous mechanistic and observational studies, in both animals and humans, further supporting a causal relationship between gastric acid-modulation and allergic sensitization.
P.A2.02 Immune development and aging from the cradle to the grave - Part 2

P.A2.02.01
CMV-infection in chronic heart failure patients contributes to a higher inflammatory status
HOSPITAL UNIVERSITARIO CENTRAL DE ASTURIAS, OVIEDO, Spain.

Chronic heart failure (CHF) is characterized by high levels of proinflammatory mediators and disease progression may be a result of the deleterious effects exerted by endogenous cytokines. Studies on the heart and the peripheral circulation. CMV-infection is the best known inducer of the differentiation of T lymphocytes in elderly and also contributes to the immunosenescence found in CHF patients. To analyze the association between CMV-serostatus and inflammation in CHF we study 40 patients (age: 55±16,9 years), 13 CMV-seronegative and 27 CMV-seropositive. Cytokine levels were quantified using a multiplex system (Luminex) and peripheral blood mononuclear cells (PBMC) were isolated and stimulated in vitro for 72 hours with anti-C3A and LPS. Higher levels of IL-1β, IL-6, TNF, IL-17A and IL-12p70 (p<0.05) were found in the serum of CMV-infected patients compared to CMV-uninfected patients. No differences in IFN-γ, IL-2, IL-4 or IL-10 levels were found. Moreover, inflammation was related not only to CMV-infection, but also to anti-CMV antibody titers which showed positive correlation with IL-1b, IL-6, TNF and IL-17A (p<0.05). When PBMC were stimulated in vitro, significant differences were also found between both groups of patients. PBMC from CMV-seropositive patients produced higher levels of proinflammatory cytokines in response to anti-C3A treatment, whereas no differences were found in response to LPS stimulation. This could be due to the greater differentiation of CD4+ and Wd+ T lymphocytes found in CMV-seropositive patients.

In conclusion, inflammation found in CHF patients may be related to dynamics of CMV-infection presumably as a consequence of their effects on T-lymphocyte differentiation.

P.A2.02.02
T-LGL leukemia cells display features of exhausted and senescent T cells
J. L. J. Assmann, M. J. Kallemijn, A. W. Langerak;
Dept. of Immunology ErasmusMC, Rotterdam, Netherlands.

Background: Large granular lymphocyte leukemia is a rare heterogeneous hematological disorder that has a chronic disease course and mostly affects the elderly. LGL leukemia is estimated to account for 2-5% of all chronic lymphoproliferative disorders. Most commonly TCRαβ+ CD8+ T cells are affected, even though in rarer cases the γδ T cell lineage can be also involved as well. Chronic (antigenic) stimulation is hypothesized to be one of the key players in disease onset. Since the disease largely presents with come of age, and since immunosenescence is a well described phenomenon in the ageing individual, here we tried to identify if T-LGL leukemia cells of both lineages present characteristics of senescence and/or exhaustion at phenotypical, functional and gene expression levels.

Results: Based on our observations through 15-color flow-cytometry and qPCR analysis, phenotypically and transcriptionally T-LGL leukemia cells were exhausted rather than senescent. On a functional level, the T-LGL cells adopted the senescence-associated secreting phenotype, which is characterized by overproduction of cytokotysis. Additionally, T-LGL leukemia cells showed diminished proliferation capacity and resistance to apoptosis, which is correlated with immunosenescence.

Conclusion: Collectively, our data indicate that T-LGL leukemia cells adopt the most detrimental characteristics from both senescence and exhaustion, thereby secreting large amounts of cytokotysis and failing in full replicative senescence, respectively. We further hypothesize that this could eventually result in the accumulation of terminally differentiated, activated, cytotoxic and apoptosis-resistant T-LGL leukemia cells that concomitantly induce heterogeneous disease characteristics in patients.

P.A2.02.03
T cell phenotypes in cytomegalovirus infected young adults are similar to those seen in elderly adults and are associated with reduced vaccine responses
G. Bowyer1, N. Venkatramani1, T. Lambe1, N. Brenner1, C. Mair1, T. Waterboer1, S. Gilbert1, A. Hill2, K. Ewer1;
1The Jenner Institute, Oxford, United Kingdom, 2Infection and Cancer Epidemiology, DKFZ, Heidelberg, Germany.

Introduction: CMV has been associated with reduced vaccine responses in both elderly and younger adults, although the underlying mechanisms are currently unclear, particularly in younger adults.

Methods: We conducted a Phase I clinical trial of viral-vectorised Ebola vaccine candidates ChAd3-EBO-Z and MVA-EBO-Z in healthy young adults (18-50 y). We assessed the impact of CMV serostatus on vaccine-specific T cell and antibody responses. We also compared T cell phenotypes in these adults in CMV- and CMV+ adults aged 60-80 years to determine the differential impacts of CMV serostatus and age.

Results: CMV seropositivity was associated with significantly reduced vaccine responses and striking differences in the global T cell repertoire with a shift towards late-differentiated memory T cells expressing CD57 and killer cell lectin-like receptor G1 (KLRG1). The proportion of vaccine-specific CD4+ and CD8+ T cells expressing these markers was significantly higher in CMV+ individuals and negatively correlated with vaccine responses - 0.71% vs 0.27% of the CD4+ and 9.4% vs 14.1% of the CD8+. CD57/KLRG1+ CD4+ T cells were expanded in both CMV+ young and CMV+ older individuals but not CMV- individuals regardless of age.

Conclusions: This study suggests that CMV, which has previously been associated with immunosenescence and reduced vaccine immunogenicity in elderly populations, can impact negatively on vaccine responses in young adults. Expansion of a subset of CD4+ T cells expressing terminal differentiation markers in association with CMV serostatus and not age suggests that CMV acquisition rather than age might be responsible for reduction of some vaccine responses in elderly cohorts.

P.A2.02.04
Landscape of naive T cell repertoire changes with human age
E. Egórov1, S. Kasatskaya1, T. Nakonechya1, M. Pogoeryly1, M. Shugay1, D. Chudakov1,2, O. Britanova2;
1IBCh, Moscow, Russia, 2Federal Medical Biological Agency, Moscow, Russian Federation.

Human aging is associated with profound changes in T cell immunity, compromising our ability to withstand the new challenges including response to infections and vaccination. These changes may further result in the imbalanced immune response leading to nonspecific inflammation provoking neurodegenerative and cardiovascular disorders, increasing risk of cancer development and autoimmune diseases. Prolonged peripheral proliferation could be associated with the functional deficiency of naive T cells that fail to differentiate towards memory phenotype upon a specific antigenic challenge. How uniform is the naive T cells proliferation on the periphery remains questionable. To shed the light on the nature of ongoing age-related changes, we focused on the comparative analysis of intrinsic characteristics of TCR repertoires for the bulk naive CD8+, bulk naive CD4+, naive RTE-enriched CD31+CD4+ and naive non-RTE CD4+ T cells derived from peripheral blood of young versus elderly healthy donors. We revealed several notable changes in characteristics of T cell repertoire. Characteristics of TCR beta CDR3 repertoires changed significantly in CD4+ and CD8+, both RTE-enriched and naive naive CD4 T cell subsets. Biochemical characteristics in the middle of TCR beta CDR3 also changed prominently. Relative publicity of CD4 but not CD8 naive T cells repertoire increased. We propose several explanations for these phenomena, and call for further studies of the mechanisms causing the observed changes and of consequences of these changes in respect of the possible wholes formed in the landscape of naive T cell TCR repertoire: this work was supported by the Russian Science Foundation project 18-10-00014.

P.A2.02.05
Progressive long-term age decline of naive but not EBV-specific CD8 T cell repertoire
B. Coutureaud1, M. Alliard2, L. Carretero-Iglesias3, D. E. Speiser1, M. Hebeisen1, N. Rufer1,2;
1Lausanne University Hospital Center and University of Lausanne, Epalinges, Switzerland, 2Ludwig Cancer Research, University of Lausanne, Epalinges, Switzerland.

Efficient T cell responses rely on the TCR-pmHC-CD8 binding ability that controls all essential T cell functions. However, it still remains unknown whether the TCR-ligand avidity is a determining factor for the clonal selection and evolution of antigen-specific T cells over time. Here, we studied the TCRββ repertoire composition and selection over a period of 15 years combined with TCR-pmHC-CD8 binding avidity analyses of large panels of CMV- and EBV-specific CD8 T cell clones. We found that the TCRββ cloneotype composition of both CMV- and EBV-specific T cell responses remains remarkably stable during the studied period. Nevertheless, within the CMV-specific cloneotype repertoires, we observed the preferential selection and expansion over time of cloneotypes of lower TCR-pmHC avidity and higher CD8 binding dependency, correlating with reduced functional capacities.

In contrast, the clonal evolution of the EBV-specific cloneotype repertoires was highly preserved, with the presence of the same cloneotype distribution (i.e. dominant versus sub-dominant, low versus high TCR avidity, CD8 binding-independend versus -dependent) over time. Our results indicate that the TCR-pmHC-CD8 binding avidity represents a major determinant of clonal selection and evolution in long-lasting CMV-specific T cells, consistent with the current concept of clonal senescence of high avidity T cells with aging. However, this is not the case for EBV-specific C8 T cell repertoires, in which the clonal composition and distribution once established is kept highly constant for at least 15 years. These findings suggest distinct mechanisms regulating the long-term outcome of CMV- versus EBV-specific CD8 T cell responses in humans.
Cigarette Smoke exposure during pregnancy could be a consequence of the inflammation process in the central nervous system (CNS) of the offspring.

A. C. S. Durão\textsuperscript{1}, W. N. Brandão\textsuperscript{1}, N. Ghodasra\textsuperscript{1}, S. J. Peto\textsuperscript{2}, T. Maroohak\textsuperscript{3}.

\textsuperscript{1}Faculty of Pharmaceutical Sciences, São Paulo, Brazil, \textsuperscript{2}Institute of Biomedical Sciences, São Paulo, Brazil.

During the implantation phase, the embryo is more vulnerable to external influences such as cigarette smoke, which can increase the risk of fetal developmental delay and immune system abnormalities. This study evaluated the effect of cigarette smoke exposure during pregnancy on an inflammatory response in the CNS of the offspring. C57BL/6 mice were exposed to 3RA4 cigarette smoke, or synthetic air, from vaginal plug to offspring birth. At the 3rd day of life, offspring were separated for the following studies: 1) In vitro: brain tissues were prepared after 3 days of treatment. After 4 days of culture, the levels of pro-inflammatory cytokines were evaluated by ELISA. As expected, the levels of TNF-α, IL-6, and IL-1β were significantly lower in the offspring exposed to cigarette smoke compared to the control group. Secondly, we evaluated the effect of cigarette smoke exposure on the expression of PD-1, a marker of T cell exhaustion, in the offspring's peripheral blood lymphocytes. A significant reduction in the expression of PD-1 was observed in the offspring exposed to cigarette smoke, indicating that cigarette smoke may have a detrimental effect on the development of Treg cells and thus contribute to the increased risk of inflammatory diseases in the offspring.

\textsuperscript{1}LUMC, Leiden, the Netherlands, \textsuperscript{2}ITMO University, St. Petersburg, Russian Federation, \textsuperscript{3}Washington University, St. Louis, United States.

Lung kinase B1 (LKB1) plays a key role in cellular metabolism by controlling AMPK activation. However, its function in dendritic cell (DC) biology has not been addressed. Here, we found that in vitro cultured murine DCs that lack LKB1 displayed impaired AMPK, but heightened mTOR activation, and expressed higher levels of costimulatory molecules and CRF upon TLR stimulation, showing higher migratory capacity, resulting in stronger T cell priming capacity in vitro and in vivo. Surprisingly however mice with a DC-specific deficiency in LKB1 (CD11c-cre / LKB1-f/f) displayed reduced effector T cell responses in models of immunization. Instead, CD11c-LKB1 mice harbored a dramatically expanded population of helios+Foxp3+ thymus-derived regulatory T cells (Tregs), already during steady state, which provides a mechanistic basis for the impaired response to immunization. Consistent with this Treg-dominated immune signature, CD11c-LKB1-mice failed to develop proper anti-tumor immunity and were resistant to allergic asthma. Mechanistically, allergic asthma in mice lacking LKB1 in their DCs displayed an enhanced ability to promote Treg differentiation which correlated with elevated antigen processing and presentation in these cells. Together, our findings identify LKB1 as a key regulator of DC activation thereby governing the development of Tregs and outcome of immune responses.

Assocation of human obesity with PD-1 exhaustion phenotype in T cells

R. Flores-Mejía\textsuperscript{1,2}, J. L. Leon-Pedraza\textsuperscript{3}, A. Monroy-Guzmán\textsuperscript{1}, O. Rodriguez-Cortés\textsuperscript{1}, R. Chacon-Salinas\textsuperscript{1}, E. Hernandez-Leon\textsuperscript{1}, E. Calderon-Austria\textsuperscript{1}, C. V. Gaona-Aquino\textsuperscript{1,2}, S. A. Estrada-Parra\textsuperscript{1,2}.

\textsuperscript{1}SEPhip, Escuela Superior de Medicina, Instituto Politecnico Nacional, México, D.F., México, \textsuperscript{2}SEPhip, Escuela Superior de Medica, Instituto Politecnico Nacional, México, D.F., México, \textsuperscript{3}Hospital General de Mexico, Ciudad de Mexico, Mexico, \textsuperscript{4}Hospital General de Mexico, México, D.F., México, \textsuperscript{5}SEPhip, Escuela Superior de Medicina. Instituto Politecnico Nacional, México, D.F., México, \textsuperscript{6}SEPhip, Escuela Superior de Medicina. Instituto Politecnico Nacional, México, D.F., México.

Lymphocytes are able to display in chronic inflammatory conditions, a diminished functional state known as exhaustion. Protein PD (Programmed Death)-1 inhibits activation and proliferation of T cells. Besides, obesity creates a chronic inflammatory state associated with insulin resistance (IR), but its influence on the development of this inflammatory state is not fully understood. In this study, we evaluated the influence of obesity and IR on the expression of PD-1 in peripheral blood lymphocytes, both CD45RO+CD4+ and CD45RA+CD4+. Obesity and IR were associated with higher expression of PD-1 in the CD45RA+CD4+ T cells, indicating an increased risk of T cell exhaustion in obese individuals. Furthermore, the expression of PD-1 was also higher in the CD45RO+CD4+ T cells from obese individuals, suggesting that the development of Treg cells may be impaired in obese individuals.

Prenatal glucocorticoid treatment, by inducing changes in the T cell receptor repertoire, has unforeseeable consequences on development of autoimmune disease. Our data showed that prenatal steroid treatment, by inducing changes in the T cell receptor repertoire, has unforeseeable consequences on development of autoimmune disease. In the NOD model, prenatal betamethasone treatment decreased the frequency of pathogenic T cells and the incidence of type 1 diabetes. In contrast, in the lupus-prone MRL/lpr strain, prenatal glucocorticoids induced changes in the T cell repertoire that led to an increased incidence of autoimmune disease. These findings suggest that prenatal glucocorticoids can have unforeseeable consequences on the development of autoimmune disease.

Methods: Time-pregnant females were treated with betamethasone one day before birth. Experimental allergic asthma was induced in C57BL/6 offspring by sensitization and challenge, and the immunological long-term consequences of prenatal glucocorticoid administration in different mouse models of disease.

Introduction: Prenatal betamethasone is routinely administered to pregnant women at risk of preterm delivery to improve survival of the newborn. Even though glucocorticoids induce a state of immunosuppression in immune cells there is evidence that they can modulate the development of the Treg cells in the immune system. Here we investigated the immunological long-term consequences of prenatal glucocorticoid administration in different mouse models of disease.

Methods: Time-pregnant females were treated with betamethasone one day before birth. Experimental allergic ashma was induced in C57BL/6 mice by sensitization and challenge with ovalbumin. The kinetics of postnatal seeding of peripheral immune cells was assessed in the same strain. The TCR repertoire and development of autoimmunity was monitored in the adult offspring of NOD (diabetes) and MRL/MpJ-Fas−/− (lupus) mice.

Results: Delayed seeding of T and B cells was observed in offspring of betamethasone-treated animals. In the NOD model, prenatal betamethasone treatment decreased the frequency of pathogenic T cells and the incidence of diabetes. In contrast, in the lupus-prone MRL/lpr strain, prenatal glucocorticoids induced changes in the T cell repertoire that resulted in more autoactive cells. No differences were observed in allergic airway response after ovalbumin sensitization and challenge in prenatally-treated animals.

Conclusions: Prenatal steroid treatment, by inducing changes in the T cell receptor repertoire, has unforeseeable consequences on development of autoimmune disease. Our data should encourage further research to fully understand the consequences of this widely used treatment.

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Aging causes loss of CD122-mediated responsiveness to interleukin-2 in CD4 T cells


Interleukin-2 is fundamental to supporting both responsiveness of effector CD8 T cells and suppression by CD25+Foxp3+ regulatory T cells (Treg). Aging has a debilitating impact on the immune system, as marked by the loss of responsiveness of Tc and impaired interleukin-2 production. Moreover, expression of CD122 among Tc and frequencies of Treg rise. Studies in young mice showed interleukin-2 to favor Tc responses when directed to the CD122 of its receptor, or to favor Treg functionality when directed preferentially to CD25 of the interleukin-2 receptor. Towards unraveling interleukin-2 functionalities at old age, we questioned whether interleukin-2 would reverse the defective Tc response at old age when favouring interleukin-2 binding to CD122 or preventing interleukin-2 binding to CD25. We supplemented anti-CD3-stimulated spleen cell suspensions of young and aged mice with interleukin-2 in the presence of antibodies blocking interleukin specifically with CD25 or CD122. Activation of T cells was measured by proliferation and expression of CD69 using flow cytometry. Our study on directing exogenous interleukin-2 to CD122 or CD25 showed that interleukin-2 promotes activation of young Tc cells via CD122, but interleukin-2 never promotes responsiveness of Tc at old age. Moreover, blocking of CD25 enhanced interleukin-2-mediated Tc activation at young age, suggesting a suppressive role for CD25. This study has many implications. This study has many implications.

Insight in dysfunctional responsiveness to interleukin-2 may improve our understanding of weakened immunity occurring among the elderly.

129

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Immunomodulation by the transcription factor FOXO3α and its pharmacological activation

J. Hartwig1, F. Sotzny1, S. Baurer2, J. Kurucz3, C. Skurt4, C. Scheibenbogen1;
1Institute for Medical Immunology, Berlin, Germany, 2University of Technology, Berlin, Germany, 3Department of Cardiology, Berlin, Germany.

Introduction: FOXO3α is a transcription factor crucial in regulation of cell metabolism, stress resistance and immunity. It is regulated by posttranslational modifications. However, mechanistic insight in FOXO3α effects on immune cell function is still limited. Therefore, the effect of FOXO3α on immune cell function as well as a pharmacological modulation of FOXO3α (e.g. metformin) will be studied in this project. Finally, different SNPs within the FOXO3α gene family for their association with aging will be analyzed regarding their functional influence on immune cells. Material & Methods: FOXO3α (p5413) was determined using Flow Cytometry (FC). Reactive oxygen species (ROS) production (DCFH-DA) and cytokine response (TNFα, IFN-γ, IL-10) was analyzed by FC. mRNA expression of FOXO3α target genes was assessed by qRT-PCR. Allelic discrimination PCR was used for FOXO3α SNP genotyping. Results: Preliminary data show an AMPK and FOXO3α activation under metformin treatment. This is confirmed by a repression of the FOXO3 target PCR2K. Immune cells treated with metformin show reduced ROS and pro-inflammatory cytokine production. Moreover, the anti-inflammatory cytokine IL-10 is increased under metformin. Conclusion: The anti-inflammatory cytokine IL-10 is increased under metformin. An association of three different gain-of-function SNPs in the FOXO3α gene, which were recently published to be associated with aging did not show a functional influence on cytokine or ROS production. Conclusion: Taken together, the results indicate that FOXO3α can be activated with metformin leading to an anti-inflammatory phenotype which might explain its anti-aging effect. DFG sponsored project

P.A2.02.12

Differential effect of cytomegalovirus infection with age on the expression of CD57, CD300a, and CD161 on T-cell subpopulations

F. Hassouneh1, N. Lopez-Setos2, C. Campos3, B. Sánchez-Correa4, R. Tarazona5, R. Solana6, A. Pera2;
1Instituto Maximiliano de Investigación Biomédica de Córdoba -Universidad de Córdoba, Córdoba, Spain, 2Immunology Unit, University of Extremadura, Cáceres, Spain.

Introduction: Immunosenescence is a progressive deterioration of the immune system with aging. It affects innate and adaptive immunity limiting the response to pathogens and to vaccines. Chronic cytomegalovirus (CMV) infection is probably one of the major driving forces of immunosenescence and its persistent infection results in functional and phenotypic changes to the T-cell repertoire. Methods: we analyzed the effect of CMV-seropositivity and aging on the expression of CD300a and CD161 inhibitory receptors and CD57 marker on CD4+ and CD8+ T-cell subsets. Results: Regardless of the T-cell subset, CD57+/CD161−/CD300a+ T-cells expand with age in CMV-seropositive individuals, whereas CD57−/CD161+CD300a+ cells decrease. Similarly, CD57+CD161−/CD300a+ T-cells expand with age in CMV-seropositive individuals in both subsets and CD57−/CD161−CD300a+ T-cells decrease in CD8+ but not in CD4+ T-cells. Besides, in young individuals, CMV latent infection associates with the expansion of CD57+CD161−/CD300a+ and CD57−CD161−CD300a+ and CD161+ T-cells. Moreover, in young individuals, CD161 expression on T-cells is not affected by CMV infection. Changes of CD161 expression were only associated with age in the context of CMV latent infection. Besides, CD300a+CD57+CD161+ and CD300a+CD161+ phenotypes were not found in any of the T-cell subsets studied, indicating T-cell senescence in CD57+ cells, CD316+ and CD300a− do not co-express. Conclusions: our results show that CMV latent infection impact on the immune system depends on the age of the individual, highlighting the importance of including CMV serology in any study regarding immunosenescence. Work supported by grant PI13/02691 from H2020/National program 2010-2013 and co-funded by “ISCIII-Subdirección General de Evaluación” and FEDER

P.A2.02.13

Investigation of intraperitoneal trafficking of lymphocytes in immunodeficient mice

R. Kugyelka1, L. Prenet1, A. Lehmann, K. Olasz, T. Berki, P. Balogh, F. Boldizsár;
1University of Pécs, Department of Immunology and Biotechnology, Pécs, Hungary.

The zeta-chain-associated protein of 70 kDa (ZAP-70) plays a key role in T cell development and signalling. ZAP-70 homozygous knockout (ZAP-70−/−) mice have no mature T cells in their peripheral lymphoid organs and blood. Previously we have shown that the adoptive transfer of wild type thymocytes reconstitutes this immunodeficiency. We have found that the intraperitoneal (i.p.) route of administration is the most effective, so we investigated the mechanism of lymphocyte trafficking after i.p. injections. We used a single i.p. injection to deliver CFSE-labeled donor thymocytes to ZAP-70−/− recipients. We sacrificed animals after various time points and investigated the cellular composition of omentum, peripheral and mesenteric lymphoid nodes, spleen. In the mesentery and omentum we analysed the localization of CFSE donor cells; we also investigated the role of various adhesion molecules (selectins, integrins) and chemokines in the process. We have found that after i.p. injection donor thymocytes leave the peritoneum and form aggregates in the mesentery along lymphoid vessels and in the omentum, however no donor-originated cells were visible in peripheral or mesenteric lymph nodes. The aggregates found in the omentum are most likely located in milky spots. Our results suggest that the omentum is an important location for thymocyte entry from the peritoneum in immunocompromised mice, as well. To gain further insight into the peritoneal trafficking we plan to investigate the possible alterations in trafficking after i.p. injection under different conditions (inflammation,stromal deficiency).

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P.A2.02.14

Characterization of human dendritic cells from elderly patients stimulated in vitro with viral proteins from hamster polyomavirus

A. Müller1, N. Maier1, S. Luth2, W. Dammermann3, K. Hanack3;
1University of Potsdam, Institute of Biochemistry & Biotechnology, Potsdam, Germany, 2Brandenburg Medical School Theodor-Fontane, University Hospital Brandenburg, Center of Internal Medicine II, Brandenburg an der Havel, Germany.

Introduction: Dendritic cells act as highly potent antigen-presenting cells and stimulate T- and B lymphocyte activation to drive immune responses. In elderly persons this potency is strongly reduced due to immunosenescence which leads to insufficient immune responses after vaccination. Increasing age is affecting innate as well as adaptive immune processes, e.g. phagocytic activity, antigen presenting capacity or antigen-specific activation of T- and B lymphocytes. To investigate this further in vitro, dendritic cells of young as well as elderly persons were stimulated with viral antigens and characterized in terms of their antigen-specific activation. Methods: Human monocytes of young and elderly persons were isolated and differentiated in vitro to naive dendritic cells using GM-CSF and IL-4. Antigen activation was performed by adding a viral antigen (VP1) in different concentrations (2.5-20 µg/mL). In vitro activated dendritic cells of both groups were analyzed and compared by flow cytometry, immunofluorescence and interleukin production. Results: Human dendritic cells could be activated antigen-specifically in vitro by using an antigen concentration of 10 µg/mL as optimum. The expression of different activation markers could be demonstrated and correlated with the production of proinflammatory cytokines. In vitro generated dendritic cells of elderly persons showed a reduced activation and capacity to stimulate naive T cells. Conclusions: There is a high clinical need for improved vaccines to treat elderly persons. The characterization of antigen-specific responses in vitro could lead to a better development of vaccine formats in the future.

P.A2.02.15

Oxidative stress and inflammation support the accumulation of highly differentiated T-cells in the bone marrow in old age and negatively correlate with Diphtheria antibody titers in the periphery

L. Pangrazzi1, E. Naismith1, A. Meryk1, K. Trieb1, B. Grubeck-Loebenstein1;
1University of Innsbruck, Innsbruck, Austria, 2University of Technology, Berlin, Germany, 3Department of Immunology, Berlin, Germany.

Aging induces a basal level of inflammation throughout the body, a condition known as "inflammaging", which contributes to immunosenescence. It has been demonstrated that memory T cells and long-lived plasma cells home to bone marrow niches, well organized structures which promote the survival of these cells. CD4+ and CD8+ effector memory T cell survival is promoted by IL-7 and IL-15 while maintenance of long-lived plasma cells is supported by APRIL and IL-6. IL-7 is important for long-lived memory T cells while IL-15 is mostly important for highly differentiated T cells, accumulation of which is associated with mortality in old age. The expression of effector memory cell and proinflammatory factors were investigated in bone marrow mononuclear cells (BMMCs) using qPCR and FACS, finding that, with age, IL-7 and APRIL decrease while IL-15, IL-6, TNF, IFNγ and IL18 increase. Incubation of BMMCs with ROS scavengers N-acetylcycteine and vitamin C reduced the levels of both cytokines in this cell population. Furthermore, proinflammatory molecules promoted the accumulation of highly differentiated CD8+ T cells, which further support inflammation. A highly differentiated cell was found between ROS, inflammation and senescent CD8+ T cells in the BM, and Diphtheria antibody titers in the serum. Our results indicate that oxidative stress and inflammation may contribute to the age-related impairments in the maintenance of immunological memory. Highly differentiated and senescent CD8+ T cells in the BM may impair the maintenance of long-lived plasma cells, leading to reduced production of antibody in the periphery as a consequence.
POSTER PRESENTATIONS

P.A2.02.16
Defective DNA repair contributes to the aging-related accumulation of a CD25+ regulatory T cell population

G. K. J. Pierer, N. A. Smits, M. E. Dölle, T. Guichelaar;
National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands.

Aging has a detrimental impact on T cells. Regulatory T cells (Tregs) accumulate with age and have therefore gained attention in studies on aging. However, the driving force behind the accumulation of Tregs has remained undefined. Gradual accumulation of DNA damage is one of the biological factors that are fundamental to the process of aging. We here aimed to define aging-related accumulation of Tregs and questioned whether defective DNA repair would drive the accumulation of Tregs.

We defined aging of Tregs in wild-type mice at young and old age, and in genetically modified young mice that express a dysfunctional form of the DNA repair protein excision repair cross-completing group 1 (Ercc1 Δ7/Δ7). Ercc1 Δ7/Δ7 is known to cause accelerated development of numerous aging-related pathologies and a reduced lifespan. We phenotypically analyzed CD4+FoxP3+ Tregs with a broad panel of aging markers by flow cytometry and defined subpopulations among these cells using cluster analysis by dimensionality reduction (ISNEL).

Multiparameter analysis revealed elevated numbers of Tregs in young Ercc1 Δ7/Δ7 mice and in old wild-type mice compared to young wild-type mice. The major subpopulation comprising this elevated number of Tregs in both naturally aged wild-type mice and in Ercc1 Δ7/Δ7 mice could be discerned uniquely by low CD25 expression. Furthermore, these CD25+ Tregs show high expression of aging-related markers PD-1 and CD44. Thus, our study shows that deficiency of DNA repair accelerates the aging-related accumulation of FoxP3+ Tregs. This accumulation is mainly due to the rise of a CD25+ subpopulation.

P.A2.02.17
Deacetylases transcript and protein expression in primary T cell in the context of aging

G. M. Tomáš, D. Quemé, B. Seliger;
1Martin-Luther University, Halle-Wittenberg, Institute of Medical Immunology, Halle (Saale), Germany, 2Martin-Luther University, Halle-Wittenberg, Institute of Anatomy, Halle (Saale), Germany.

The capacity of the immune system to protect the organism declines with age, this is observed in the increased susceptibility to infections and decreased efficiency of vaccination.

Posttranslational modifications of proteins (PTMs) contribute to this by the accumulation of misfolded proteins. One of the most abundant PTMs is acetylation. This process is regulated by: acetyltransferases (ex: p300) and deacetylases. Deacetylases are historically called histone deacetylases (HDACs), but their range of substrates is much wider. The 3rd class of deacetylases is called Sirtuins (SIRT). SIRT 3 and 6 have been shown to have a role in the aging process. Our aim is to analyze if the deacetylases expression levels change during aging. For this purpose, CD4+ and CD8+ T cells have been sorted magnetically from PBMCs of healthy blood donors from 2 age groups (<30 yo and >60 yo) and have been stimulated with plate bound antiCD3 and soluble aCD28 for 48h, then the transcript expression of HDACs and Sirtuins has been assessed by qPCR and the protein expression by flow cytometry. Additionally, the constitutive transcript expression of CD4+ and CD8+ T cells has been compared to non-T cells. Sirtuin expression was found higher in T cells, while Sirtuin and p300 expression was decreased in aged CD4 T cells. These results will be linked to proliferation assays and flow cytometry analysis of the T cell subsets. The results of our study will provide insights into the role of deacetylases in the aging T cell.

P.A2.02.18
Comparative analysis of B-cell repertoires induced by live yellow fever vaccine in young and middle aged donors

M. A. Turcchanina, A. N. Dayakov, A. S. Obratsova, M. Lebedin, D. Staroverov, E. M. Merysik, O. V. Britanova, D. M. Chudakov, A. Z., T.; 1Institute of Bioorganic Chemistry, Moscow, Russian Federation, 2CEITEC, Masaryk University, Brno, Czech Republic, 3Bjornow State University, Moscow, Russian Federation, 4Skolkovo Institute of Science and Technology, Moscow, Russian Federation.

Aging is associated with a dysregulation of immune function and age-related changes are reported in many B cell populations. These changes include attenuated response to vaccines, an exhausted immune repertoire displaying a substantially lower diversity of T cell receptor as well as impaired antigen-driven selection mechanisms. Recent advances in next-generation sequencing technology have allowed us to perform high-resolution characterization of the antibody repertoire, and of the changes that occur following vaccination in different age donors. In present study we employed 5’RACE UMI-based full length error-free immunoglobulin profiling to compare plasma cell antibody repertoires in young and old donors vaccinated with live yellow fever vaccine. Our analysis has revealed age-related differences in the responding antibody repertoire ranging from distinct IGH CDR3 repertoire properties to differences in somatic hypermutation profiles and antibody lineage tree structure. Young vaccinated individuals respond with a repertoire containing significantly longer CDR3, implying potentially higher sequence diversity. Elder individuals tend to respond to a new challenge with IGH variants carrying shorter CDR3s with higher content of hydrophobic and strongly interacting amino acid residues. Clonal lineage structure analysis reveals that elder individuals have higher total number of newly acquired hypermutations. But at the same time in elder individuals, replacement-to-silent ratio among the newly acquired unique somatic hypermutations was significantly lower compared to the young donors. Overall, our findings suggest that younger individuals respond with a more diverse antibody repertoire and employ a more efficient somatic hypermutation process than elderly individuals in response to a newly encountered pathogen.

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P.A2.02.19
Resting calpain activity as the key factor in human T cell genesis, function and aging

1Department of Pathophysiology, Medical University of Gdańsk, Gdańsk, Poland, 2Department of Pathology and Experimental Rheumatology, Medical University of Gdańsk, Gdańsk, Poland, 3Department of Clinical and Social Gerontology, Medical University of Gdańsk, Gdańsk, Poland, 4Department of Pediatric Cardiac Surgery, Pomorski TraumaCenter, Gdańsk, Poland, 5Faculty of Medicine and Health Sciences, Research Center on Aging, Graduate Program in Immunology, University of Sherbrooke, Sherbrooke, QC, Canada.

Introduction: Calpains and their inhibitor calpastatin form a self-regulatory calpain-calpastatin system (CS) of limited proteolysis controlling proliferation and apoptosis. Their role for proliferation and aging of human T cells and for thymocyte differentiation was assessed here together with some mechanistic aspects of their maintenance and function.

Methods: Peripheral blood mononuclear cells were obtained from healthy young, elderly and centenarians, while thymocytes were obtained from infants undergoing heart surgery. Cells were left untreated or treated with specific membrane-permeable calpain inhibitors. Lymphocyte proliferation was assessed by flow cytometry using VPD450 dividing cell tracking. Amounts and activities of calpains, as well as the amounts of calpastatin and of phosphorylated NFκB, PLCγ, and p56Lck were assessed in different T cell and thymocyte populations by flow cytometry. Expression of the CS genes and of some relevant miRNAs identified by the NGS was performed by qRT-PCR.

Results: Calpains are differently active in all populations of T lymphocytes and thymocytes. In the periphery, this activity is necessary for proliferation and cytokine secretion and associated with adequate phosphorylation of signaling molecules. It is sustained by constitutive expression of the CANP-1, CANP-2 and CAST genes correlated with certain mirRNAs. With aging, the amounts and activities of calpains change in parallel with impaired proliferation and cytokine secretion, but are maintained at “young” levels in the centenarian T cells.

Conclusion: Constitutive activity of calpains in resting thymocytes and T cells seems indispensable for appropriate differentiation and function of these cells and is modified during aging.

P.A2.02.20
The strategy of the human memory B cell response changes throughout life

R. Carsetti, E. Piano Mortari, G. Grimsholm, A. Aranburu;
1Bambino Gesù Children Hospital IRCCS, Rome, Italy, 2Department of Rheumatology and Inflammation Research, Gothenburg, Sweden.

Immunological memory, including memory B cells (MBCs), plasma cells (PC) and their antibodies, is generated by the reaction to infection and vaccination, and protects us from re-infection. Two populations of memory B cells have been described, switched and IgM memory B cells, that execute different and non-interchangeable functions. We have shown that before, whereas switched memory B cells are mostly generated in the germinal centers at all ages, IgM memory B cells can be distinct in three types with different developmental history: innate, remodelled and germinal-center-derived IgM memory B cells. CD27 is the cell surface marker able to identify most MBCs in human. The intensity of CD27 expression changes with age. We now show that, independently of the expressed immunoglobulin isotype, in infants MBCs express low levels of CD27 (CD27−/−), whereas switched memory B cells are CD27+ and CD27+Bright MBCs represent two distinct and sequential MBC development stages. Stringent Ag-driven pressure selects CD27+Bright into the CD27bright IgM B cell pool. Our results identify the actors and the strategy of the human MBC response throughout life and give the rationale for the design of age-tailored vaccination protocols.
It is a central theme in thymus biology to understand how temporally dynamic molecular mechanisms control thymic T cell development in vivo. It is therefore important to clarify the temporal sequences of thymic T cell differentiation, but this is difficult due to a lack of tools to analyse the temporal dynamics of differentiating T cells. Since thymic T cell differentiation is driven by T cell receptor (TCR) signalling, we recently established a new tool, Timer of cell kinetics and activity (Tocky) and developed a Timer reporter for a TCR downstream gene, Nr4a3. Timer protein spontaneously changes emission spectrum from blue to red, enabling investigations of the temporal dynamics of molecular and cellular events following TCR signalling in vivo.

We first determined the decay rates of Timer-blue and -red fluorescence in order to establish Nr4a3-Tocky as a quantitative tool to investigate TCR signal dynamics. Timer-blue fluorescence had a half-life of 4 hours, while Timer-red one was over 5 days. Therefore, blue fluorescence reports real-time transcription, while red fluorescence reports transcriptional history. We then used Nr4a3-Tocky to study thymic T cell development. Within CD4⁺CD8⁺ double-positive cells, Timer blue expression occurred mostly in the CD69⁺ fraction. In CD4⁺ and CD8⁺ single-positive cells, Timer expression was reported by GITR⁺ cells. Finally, we show the temporal sequences of differentiation events for regulatory T cell differentiation using multidimensional analysis.

In conclusion, we establish Nr4a3-Tocky as a new tool for analysing the temporal dynamics of cellular differentiation, and using this, we demonstrate how T cells develop across time during selection processes in vivo.

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P.A2.02.11  
In vivo dynamics of thymic T cell differentiation revealed by a new Timer approach

A. D. Paduraru, T. Bending, T. Crompton, M. Ondzi
1Imperial College London, London, United Kingdom 2University College London, London, United Kingdom

The thymus is the primary lymphoid organ where naïve T cells are generated, however, with the exception of age, the parameters that govern its function in healthy humans remain unknown. Herein, we characterized the variability of thymic function among 1,000 age- and sex-stratified healthy adults of the Milieu Intérieur cohort, using quantification of T cell Receptor (TCR) Effector Circles (TRECs) in peripheral blood T cells as a surrogate marker of thymopoiesis. Age and sex were the only non-heritable factors identified that impact thymic function. TREC levels decreased with age (5% per year, P=3x10⁻⁸) and were higher in women compared to men (66% increase, P=2x10⁻¹⁴).

P.A2.02.22  
The role of Nr-kappaB transcription factors in virtual memory (Tvm) cells

D. P. Ellis, T. Fulford, R. Grumont, R. Slattery, S. Gerondaki
1Monash University, Melbourne, Australia 2Peter Doherty Institute, Melbourne, Australia

Virtual memory (Tvm) cells are a subset of memory phenotype T cells that arise naturally in naïve, lympho-replete mice. In contrast to conventional memory CD8 T cells that are generated following an antigen-dependent T cell effector immune response, Tvm cells develop from naïve CD8 T cells in response to cytokine-dependent homeostatic proliferative signals, in particular interleukin-15 (IL-15).

Utilizing two murine models, we show that the NF-κB transcription factor RelA plays a cell-intrinsic role in the homeostatic maintenance of Tvm cells. Lethally irradiated mice reconstituted with RelA-/- foetal liver-derived hematopoietic stem cells (HSCs) fail to maintain a RelA⁺ Tvm cell population. This phenotype, which appears in part due to an inability to compete with residual (RelA⁺) Tvm cells for survival/proliferation signals co-expressed with reduced expression by RelA-/- Tvm cells of the IL-2/15 receptor β-chain (CD122). Conditional inactivation of RelA in T cells (Lck-CreRelA conditional mice) reveals an issue in maintenance with these mice exhibiting a decline in the Tvm cell population from 8 weeks of age to ~50% of normal numbers by 12 weeks, after which this reduced level of Tvm cells is maintained throughout adult life. In addition to a reduced expression of CD122, IL-7 receptor α-chain (CD127) expression is lower on Lck-CreRelA Tvm cells when compared to age-matched littermate controls (Lck-CreRelA⁻ mice), a finding suggestive of an IL-7 survival defect. Collectively, these results indicate RelA controls the homeostatic maintenance of Tvm CD8 T cells by regulating the expression of cytokine receptors important for Tvm cell survival and division.

P.A2.03 Immune development and aging from the cradle to the grave - Part 3

P.A2.03.01  
Follicle lymphoid aggregates (FLAGs) - a novel member of serosal lymphoid organoids in mice

B. Bologh, J. Kinkal, O. Jacobsen, G. Bedics, B. Botz
1Department of Immunology and Biotechnology, Pécs, Hungary 2Department of Pharmacology and Pharmacotherapy, Pécs, Hungary 3Molecular Pharmacology Research Group, SzentGyörgyi Research Center, Pécs, Hungary

The involvement of non-mucosal lymphoid compartments of the abdominal cavity in the systemic immune responsiveness is almost completely unexplored. In addition to the milk spots within the omentum (MS), small congregates of leukocytes have been described in adipose tissue (fat-associated lymphoid clusters – FALCs). Here we report the identification of a novel form of serosal lymphoid organoids that can efficiently collect intraperitoneal B cells and B-lymphoma cells.

Using a spontaneous high-grade B-cell lymphoma a novel set of lymphoid tissues has been observed, which is characterized by a follicle appearance linked to visceral fat and omental adipose tissue as well as peritoneal membrane, denoted as Follicle Lymphoid Aggregate (FLAG). Their tissue architecture and developmental requirements were studied using whole-mount immunohistochecmistry revealing how an early macrophage-rich condensation followed by gradual enrichment of B cells, leading to FLAG-like transformation. In Rag-/- mice these structures are absent, whereas the transfer of purified B cells restores them. B cells show a partial segregation from T cells that appear to accumulate in the central regions. These FLAg structures are initially demarked and encapsulated by a rim of macrophages displaying LYVE-1 antigen, and are sensitive for clodronate liposoma-mediated depletion, which also blocks subsequent B cell entry. In vivo bioimaging tracing reveals that both normal B cells and high-grade B-cell lymphoma cell efficiently home upon intraperitoneal transfer. These data reveal the existence of novel visceral lymphoid tissue formations which may influence abdominal immune surveillance.

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P.A2.03.02  
Early dynamics of mucosal NK cells in the small intestine of infants

A. Sagebiel, F. Steiner, S. Lunemone, C. Koerner, S. Schreuer, M. Altfeld, D. Perez, K. Reinhagen, M. J. Buddens
1Heinrich-Pette Institute, Hamburg, Germany 2Institute of Medical Microbiology, Hamburg, Germany 3Department of Cancer Immunology, Genentech, South San Francisco, United States

The involvement of non-mucosal lymphoid compartments of the abdominal cavity in the systemic immune responsiveness is almost completely unexplored. In addition to the milk spots within the omentum (MS), small congregates of leukocytes have been described in adipose tissue (fat-associated lymphoid clusters – FALCs). Here we report the identification of a novel form of serosal lymphoid organoids that can efficiently collect intraperitoneal B cells and B-lymphoma cells.

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P.A2.03.03  
Human thymopoiesis is controlled by a common genetic variant within the TCR-TCRD locus

E. Clove1, I. Leston Araujo1, C. Alania1,6, E. Patini1, J. Bergstedt, A. Urutu1,4, S. Lopez-Lstra2, Y. Li3, B. Charbit, M. Hasan, C. R. MacPherson, B. L. Melo-Lima,4, C. Dauvoy1, N. Saut2,6, M. Germani6,4, D. Trepouet1,4, P. Morange1,6,4, M. Fontes1,4,6, D. Duffy1,4,6, J. P. Di Santos1,4, L. Quintana-Muro1,4, M. L. Albert1,4, A. Toubert1,4, The Milieu Intérieur Consortium
1INSERM UMR1 1160, Paris, France 2Université Paris Diderot, Sorbonne Paris Cité, Paris, France 3Center for Translational Research, Institut Pasteur, Paris, France 4Dendritic Cell Immunobiology, Institut Pasteur, Paris, France 5INSERM UMR1 1223, Paris, France 6Human Evolutionary Genetics, Institut Pasteur, Paris, France 7CIMRS UMR0-2000, Paris, France 8Center of Bioinformatics, Biostatistics and Integrative Bioinformatics, Institut Pasteur, Paris, France 9Department of Automatic Control, Lund University, Lund, Sweden 10Innate Immunity Unit, Institut Pasteur, Paris, France 11Laboratory of Haematology, Le Timone Hospital, Marseille, France 12Aix Marseille University, INSERM UMR1-226, INRA U1260, Center for Cardiovascular and Nutrition Research (C2VN), Marseille, France 13Sorbonne Université, Université Paris 6 / UPMC, INSERM, UMR1-1166, Genomics & Pathophysiology of Cardiovascular Diseases, Paris, France 14Institute for Cardiometabolism and Nutrition (ICAN), Paris, France 15International group for data analysis, Institut Pasteur, Paris, France 16Department of Cancer Immunology, Genentech, South San Francisco, United States 17Laboratoire d’immunologie et d’Histocompatibilité, Hôpital Saint-Louis, AP-HP, Paris, France

The thymus is the primary lymphoid organ where naïve T cells are generated, however, with the exception of age, the parameters that govern its function in healthy humans remain unknown. Herein, we characterized the variability of thymic function among 1,000 age- and sex-stratified healthy adults of the Milieu Intérieur cohort, using quantification of T cell Receptor (TCR) Effector Circles (TRECs) in peripheral blood T cells as a surrogate marker of thymopoiesis. Age and sex were the only non-heritable factors identified that impact thymic function. TREC levels decreased with age (5% per year, P=3x10⁻⁸) and were higher in women compared to men (66% increase, P=2x10⁻¹⁴).
In addition, genome-wide association study revealed a common variant within the T-cell receptor TCRα-TCRβ locus, between the D02 and D03 gene segments (rs2204985, P=1.9x10^-17), which associates with variable TREC numbers. Strikingly, transplantation of human hematopoietic stem cells with the rs2204985 GG genotype into immunodeficient mice led to thymopoiesis with higher TREC levels, increased thymocyte counts and a higher TCR repertoire diversity. Our population immunology approach revealed a genetic locus that controls thymopoiesis in healthy adults, with potentially broad implications in precision medicine.

**P.A.2.03.04**

**Accelerated aging in the immune system in childhood and adolescent neuroblastoma survivors**

P. Burilova1, K. Bendickova1, S. S. Jose1, T. Kepak1, Z. Krenova1, J. Fick; 1Center for Translational Medicine (CTM) Clinical Research Center (ICRC), Brno, Czech Republic, 2Pediatric Hematology and Oncology, Clinical University Hospital Brno, Brno, Czech Republic, 3Pediatric Oncology Translational Research (POTR), International Clinical Research Center (ICRC), St. Ann’s University Hospital Brno, Brno, Czech Republic, 4Pediatric Oncology, Translational Research (POTR), International Clinical Research Center (ICRC), St. Ann’s University Hospital Brno, Brno, Czech Republic.

Senescence of immune cells is characterized by the decline of immune functions including adaptive as well as innate responses and is associated with a number of pathologies linked to aging, including a higher susceptibility to infections or cardiovascular diseases. While senescence of adaptive immunity is relatively well described, mechanism of aging-related senescence in myeloid cells is only poorly understood. Senescence progression has been associated with several chronic inflammatory disorders but more interestingly has been observed in survivors of cancer therapy. The intensive therapeutic approach generates negative burden for patients’ immune system, fueling persistent sterile chronic inflammation. Post-treatment sequelae of cancer therapy or severe tissue damage, called damage-associated molecular patterns (DAMPs), often accumulate within the adjacent tissue and eventually spread within the blood stream. Myeloid cells and their progenitors are capable of binding DAMPs via TLRs, this results in pro-inflammatory cytokines production and initiation of inflammation. We address the impact of long-term administration of 13-cis-retinoic acid and topotecan currently available for neuroblastoma therapy, and DAMPs triggers to accelerated onset of immunosenescence caused by persistent low-grade inflammation. Distribution of monocytes into subsets and phagocytic activity from patient samples with neuroblastoma: the putative extra-germinal somatic mutation in early childhood malignancies. Further, we test the possible impact of mentioned triggers to hematopoiesis is tested in vitro using human induced pluripotent stem cells derived myeloid cells. This project aims to develop new prognostic markers allowing to assess the progression of immunosenescence in order to prevent further serious complications, which occur long-term after the successful therapy.

**P.A.2.03.05**

**T-bet+ CD8αα TCRαβ intraepithelial lymphocytesprecursor progress through a PD-1 stage and depend on C-myc**

J. Hummel1, K. Ebert2, J. Fixemer, Y. Tanriver; 1University Medical Center, Freiburg I.B., Germany.

Introduction: IELs (IELs) are a heterogeneous resident T cell population within the epithelial barrier in the small intestine and can be divided into natural (CD8αα TCRαβ or TCRγδ) and induced (CD8αβ TCRαβ). Thymic IEL precursors (IELps) of natural CD8αα TCRαβ IELs are post-selected T cell receptor positive (TCRαβ+) T cells that lack expression of the classical co-receptors CD4 and CD8 (double negative, DN) and NK1.1, which distinguishes them from natural killer T cells. We could recently refine this definition by demonstrating that lineage restriction in IELps towards DN TCRαβ cells is gradually imposed by the T-box transcription factor T-bet. Here, we demonstrated by employing a novel developed PD-1 fate map and conditional T-bet knockout mice that all natural IELs progress through a PD-1- stage, that is gradually lost upon T-bet expression. This transition is regulated by the transcription factor C-myc, as it cell-specific conditional C-myc knockout mice lack T-bet+ IELps, while PD-1+ IELps are still present. Using C-myc+/-OTI-OVA chimera as a model of agonist-selected-IELps, we could show that high avidity antigen cannot compensate for the loss of C-myc in IELps. Thus, the loss of C-myc does not affect the antigenic selection of the IEL population leading to DN TCRαβ/NK1.1 IELps but lacking the final matured T-bet+ IELp. Hence, the pronounced upregulation of C-myc during thymic IEL development is essential for the induction of t-bet, which in turn regulates proliferation and differentiation.

**P.A.2.03.06**

**Immunization-induced thymic atrophy as a contributing factor in strain differences in rat susceptibility to EAE**

M. Nacks-Aleksic1, M. Stojanovic1, I. Pilipovic1, D. Kosev1, G. Leposovic1; 1Department of Physiology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia, 2Immunology Research Centre “Branislav Janković”, Institute of Virology, Vaccines and sera “TORLAK”, Serbia, Belgrade, Serbia.

Introduction: It is stated that impaired thymopoiesis in autoimmune diseases contributes to their perpetuation. To prove this hypothesis, influence of immunization on EAE on thymopoiesis and the putative thymic-dependent changes in the periphery were examined in susceptible (Dark Agouti, DA) and resistant (Albino Oxford, AO) rats. Methods: DA and AO were immunized with myelin basic protein (MBP) on days 1 and 10 of life, and the expression of thymic precursors (IELps) and mature T cells and regulatory CD4+Foxp3+CD25+ cells (nTregs) on thymocytes, their apoptosis and proliferation, frequency of recent thymic emigrants (RTEs) and CD28-α4 cells in CD4+ and CD8+ peripheral blood lymphocytes (PBLs), and thymic expression and circulating levels of cytokines influencing thymus/thymopoiesis were investigated. Results: In rats of both strains increase in proinflammatory-cytokine circulating levels followed by thymic atrophy and changes at multiple thymopoietic developmental points, leading to decreased number of the most mature CD4+ and CD8+ TCRαβ+ thymocytes and frequency of RTEs among PBLs (as in chronobiological aging), was found. This was more prominent in DA rats. Consistently, compared with AO rats, in DA rats were found higher frequencies of cytotoxic CD28+ cells (contrasting to target tissue damage) among CD4+ PBLs and cytotoxic granyme B+ CD4+ T cells in spinal cord. Additionally, compared with non-immunized controls, DA rats exhibited greater decline in thymic nTreg generation (reflecting diminished thymic IL-7, IL-2 and IL-15 expression) than AO ones. Conclusions: The study suggests that differences in thymopoiesis, and consequently nTreg and CD28+CD8αβ T cell frequency in the periphery, contribute to strain differences in EAE clinical presentation. (Grant 175050, MESTO, Republic of Serbia).

**P.A.2.03.07**

**INF-g and TNFα serum concentrations and frequency of INF-g and TNFα genotypes in children with primary immunodeficiency and recurrent respiratory tract infections (without significant immunological abnormalities)**

A. Lewandowicz-Ustynska1, G. Pasternak1, K. Bogunia-Kubiś2; 1Wrocław Medical University, Wrocław, Poland, 2Provincial Hospital J. Grąmekowski, Wrocław, Poland, 3Polish Academy of Sciences, Wrocław, Poland.

The aim of the study was to evaluate the concentrations of INF-g and TNFα in children with primary immunodeficiency (PID). The studied included 93 children: 30 patients with PID, 43 RTI, 20 healthy children (control group). The concentrations of INF-g, TNFα, and INF-g and TNFα polymorphisms were determined. The polymorphism of the TNF-α interleukine (IFN-γ; rs1800629 - 308 G/A) and IFN-gamma (IFNG; rs2430561 +874 T/A) were determined. Genotyping was done by melting curve analysis. This method is based on real-time PCR, i.e., PCR where the detection of amplified DNA can be made during the reaction without the need for separated detection after the reaction. LightSNP Assay (TIB MOLBIOL) and Probes Master Mix (Roche) were used for typing. Amplification was performed on LightCycler® 480 MultiWell Plate 96 plates on the LightCycler® 480 II (Roche). The research was carried out in the Laboratory of Clinical Immunogenetics and Pharmacogenetics of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wrocław. No statistically significant differences in serum INF-g, TNFα, serum levels were observed in the examined children. The frequency of genotypes TNFα and IFNG in the group of patients (N =93); INF-g (rs1800629 - 308 G/A) in all studied groups was the rarest GA, the most common GG. IFN-gamma (IFNG; rs2430561 +874 T/A) in all studied groups was the rarest genotype AA, the most common AT.

**P.A.2.03.09**

**Chronic disease development increases with suppression of acute infectious diseases in the population - a case controlled pilot study of 166 cases**

S. Mohebi1, M. Mallappa2, G. Vithoulkas3; 1Centre For Classical Homeopathy, Bangalore, India, 2University of the Aegean, Alonissos, Greece.

Introduction: The role of the world’s leading cause of morbidity and mortality from infectious disease over the past few decades cannot be attributed to the increase in hygiene or change in lifestyle alone. This raises the question - whether the manner in which immune system responds in response to suppression of acute inflammation is detrimental to the general health of the population. This study aimed to test the hypothesis that the development of chronic diseases increases with suppression of acute infections in the population.

Materials and Methods: Teenagers opting for treatment at Centre For Classical Homeopathy, Bangalore, India were analysed for their medical history and categorised into 4 groups Group 1: Present acute infections only - recurrent or not. (control group) Group 2: Present acute infections - at least one in the past 1 year with present chronic diseases diagnosis. Group 3: Present chronic disease diagnosis with history of recurrent infections in the past. Group 4: Present chronic disease diagnosis with no history of recurrent infections in the past.

Results: The trend of the chronic diseases was to cluster in group 4. The most prevalent 3 chronic conditions viz., allergic rhinitis, allergic rinitis and chronic headaches also showed a tendency to cluster in group 4.

Conclusions: This study shows that there is a tendency for chronic diseases to be significantly higher in people without acute infections. This data may be used further to evaluate the role of different treatment modalities that suppress acute inflammation in the body.
A network of transposable elements controls CD4 T cell fate

A. Molbec*, V. Adoue*, B. Binet*, J. Fourquet, J. Joffre; CPFP, Université de Toulouse, CNRS, Inserm, UPS, Toulouse, France.

CD4 T lymphocytes are highly efficient at protecting the host against endogenous and exogenous dangers. Their efficiency comes at least in part from their ability to adapt their phenotype and function to the threat detected by the cells of the innate immune system. T helper cell differentiation and commitment are strictly controlled by epigenetic mechanisms. They are necessary to establish lineage-specific gene expression programs while repressing genes associated with alternative fates. Combining genome-wide transcriptionomic and epigenetic studies with functional genotyping and transgenic modeling, we have identified a novel T cell regulatory pathway to help inform the use of these cells in cancer therapeutics.

Do thymic γδ T cells count on the antigen receptor for effector differentiation?

S. Medrano-García1, A. V. Marín1, H. De La Figuera1, J. R. Regueiro2, M. Muñoz-Ruíz1, E. Fernandez-Malavé1,2,3; 1Complutense University, Madrid, Spain, 2Hospital 12 de Octubre Research Institute (imas12), Madrid, Spain, 3The Francis Crick Institute, London, United Kingdom.

Whether the T cell receptor (TCR) is critical for the generation of γδ T cell subtypes in the thymus is unclear. A popular view proposes that TCR signal "strength", which is normally dependent on surface TCR expression, is a major determinant of the generation of γδ T cells producing either IFN-γ or IL-17. We have recently reported that Cδδγ- Cδδγα (CD3DH) mice had reduced surface TCR expression and signaling in thymic γδ T cells, and exhibited a marked depletion of IFN-γ-producing γδ T cells. Here, we have revisited the TCR signal strength model using CD3δ KO mice, whose surface TCRγδ levels are higher than in CD3DH but lower than in WT. We also analyzed CD3δ/Cδδγ+ doubly-KO mice expressing a human CD30 transgene (hDTg), which display consistently higher surface TCRγδ than WT. Along the developmental pathway of IFN-γ-producing γδ T cells (defined by sequential CD122 and NK1.1 expression), CD3δ KO showed a markedly increased CD122+/NK1.1+ compartment and CD122+/NK1.1+ cells with reduced surface NK1.1, when compared to WT. These two subsets were comparable in frequency and surface NK1.1 in hDTg and WT mice. Anti-CD3 i.p. injection reversed the apparent blockade at the CD122+/NK1.1+ stage of CD3δ KO. On the other hand, the relative abundance of putative IL-17-committed γδ T cells (CD27-CCR6−) was reduced in CD3δ KO but augmented in hDTg mice, particularly in a unique population displaying the highest TCR levels. Our study supports the existence of distinct TCR expression/signaling requirements for effector γδ T cell differentiation in the thymus.

Dielectric properties of serum in children with recurrent respiratory tract infections

G. Postermal1, D. Luczycka1, K. Penta1, K. Gu1, M. Karmierowska-Niemczak1, A. Lewandowicz-Uzyniak1,2,3; 1Wrocław Medical University, Wrocław, Poland, 2Provincial Hospital J. Gromkowski, Wrocław, Poland, 3Wrocław University of Environmental and Life Sciences, Wrocław, Poland.

Despite the improvement of living conditions, availability of medicines and various methods of treatment, recurrent respiratory tract infections continue to occur and are a frequent clinical problem of children of our climate zone (mild winter intermezzo nature). It results, among others, from the geo-climatic conditions, lifestyle, and maturation of the immune system (especially in human immaturity in developmental age). One of the reasons for these ailments is a variety of abnormalities in the immune system that may have of primary or secondary nature. We are still looking for new conditions that may underlie recurrent infections. Various screening assays are also tested to demonstrate quickly and easily abnormalities in selected parameters of the immune system, so that detailed, cost-intensive and time-consuming immunoassays can be performed in justified cases.

The aim of the study was to examine the occurrence of dependence between selected physical parameters of serum such as: electrical conductivity, electrical permeability, dielectric loss factor, blood tests results in the following: blood counts, serum glucose concentration, micronutrient concentrations, and selected parameters of the immune system: concentrations of the main classes of immunoglobulins (IgG, IgA, IgM and IgE), complement hemolytic activity, and neutrophil function in patients suffering from recurrent respiratory tract infections compared to those without recurrent infections. During the impedance spectroscopy of the tested samples, there was a differentiation of the obtained measurement results. In order to develop the obtained measurement and analytical data, a correlation matrix was determined and a grouping of the analyzed cases was carried out.

Phenotypic characterization of immune cells from premature neonates with extreme low birth weight

K. Qazi Rahman; Stockholm University, Stockholm, Sweden.

Background: The development of the immune system begins during the first trimester, but continues to develop after birth. Premature children have a compromised immune system with regard to both quantitative and qualitative aspects. There is an increased risk of sepsis, necrotizing enterocolitis and pneumonia—all common causes of mortality, primarily in the group of neonates with extremely low birth weight (ELBW).

Objective: To characterize innate and adaptive immune compartments of ELBW infants 14 days after birth.

Materials and methods: Peripheral blood mononuclear cells (PBMC) were collected 14 days after birth from 79 ELBW premature infants, participating in a randomized double-blind placebo-controlled study of probiotic supplementation. As a control, PBMCs from 29-term (FT) infants at 14 days of age were used. Mononuclear cell populations were analyzed by multi-colour flow cytometry.

Results: The proportions of CD4 and CD8 T cells, regulatory T cells, NK cells and monocytes were significantly lower in PBMC of ELBW premature infants compared to FT infants. On the contrary, γδ T cell- and NK cell frequencies were comparatively high in the ELBW infants. Further, an elevated CD4+CD8 ratio was observed in ELBW premature infants. The expression levels of homing receptors CCR4 and CCR9 on T cells were high, while monocyte expression of HLA-DR and CD68 was very low in ELBW infants.

Conclusion: We provide an extensive characterization of the peripheral immune compartment of ELBW premature infants that provides important information for future studies on immune function and possible therapeutic interventions.
CONCLUSION: Our study provides a novel model for CD8 transcription factors, Stat1 and Stat4, that T CD45RA and CCR7 for analysis of open chromatin by ATAC-seq, for transcriptional analysis by microarray and for phenotypic and functional assays.

METHODS: CD8+ T cells were isolated from Tdap-vaccinated/unvaccinated pregnancies: maternal at booking (B-21weeks), maternal and cord at birth, and infants at 7weeks and Smonths (pre- and post-their primary immunisations). Antibody levels directed against pertussis vaccine antigens (Prn, FHA, PTx, DTx, TTx) were measured using a fluorescent bead-based multiplex immunoassay. Cellular responses to Bordetella pertussis were measured by whole blood stimulations with strains of heat-killed pertussis (wild-type, Prn-negative, Prn/ FHA-negative) followed by multiplex cytokine analysis.

RESULTS: Maternal Tdap vaccination significantly increases vaccine-specific antibody titres in women infants before primary immunisation. Post-childhood vaccination antibody titres were the same between babies born to vaccinated and unvaccinated pregnancies by 5 months of age. Cytokine responses to Bordetella pertussis were seen, that may be altered in infants from vaccinated pregnancies.

Conclusion: Maternal pertussis vaccination boosts antibody prior to the first dose of infant vaccination. There was no impact on infants’ antibody response to DTaP vaccination. Like adults, infants mount strain-dependent cytokine responses to B.pertussis that are not impacted by maternal vaccination. Age-dependent effects are under investigation.

P.A2.03.16

Age-related defects in effecorycytosis are reversed by inhibiting p38 MAPK activity in aged volunteers

R. C. van de Merwe4, R. P. De Mooyer1, A. N. Akbar1, D. W. Gilroy4;
University College London, London, United Kingdom.

Ageing is associated with chronic inflammation, which may be due to failed resolution. As p38 MAPK regulates pro-resolution pathways including effecorycytosis, we investigated p38 MAPK signaling in aged versus young humans using a skin blister model of self-limiting acute inflammation. Thereafter, we used the p38 MAPK inhibitor losmapimod to discern p38 MAPK’s role in the onset and resolution of inflammation in the aged. Cantharidin (0.1%) was applied topically to young (<40 years) and aged (>65 years) human volunteers. A cohort of aged volunteers were exposed to losmapimod (15mg BID/PO) for 4d prior to cantharidin application. Blister exudates were collected at 24+72h and were analysed by flow cytometry. Phospho-p38 MAPK was increased in HLA+DR+ blister cells in aged compared to young volunteers. In aged volunteers, losmapimod early apoptotic cells accumulated at the 72h resolution time point; a finding not observed in young volunteers. Furthermore, PMN clearance in aged volunteers (losmapimod) did not correlate with CD14+ mononuclear phagocyte (MPs) numbers in contrast to young volunteers. This indicated failed apoptotic body clearance in the aged, which we hypothesise arises from defective effecorycytosis. Aged MPs expressed lower TIM-4, a molecule involved in apoptotic body clearance, compared to younger counterparts. In aged volunteers losmapimod elevated TIM-4 expression on MPs, increased MP numbers at resolution, and rescued the correlation between MP numbers and PMN clearance. Inhibition of p38 MAPK in aged humans alters the cellular profile of cantharidin blisters, and may enhance effecorycytosis during the resolution of inflammation by increasing TIM-4 expression on MPs.

P.A2.03.17

Homeostatic cytokines revert primed CDB+ T cells to naïve-like memory stem variants through epigenetic and transcriptional reprogramming

G. Frumento4, K. Verma1, W. Croft1, A. White1, Z. Nagy1, S. Kissane1, S. P. Lee4, G. Anderson4, P. Moss4, F. E. Chen1,2,3;
1Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom, 2NHS Blood and Transplant, Birmingham, United Kingdom, 3Centre for Computational Biology, University of Birmingham, Birmingham, United Kingdom, 4Institute of Immunology and Ageing, University of Birmingham, Birmingham, United Kingdom, 5Technology Hub, University of Birmingham, Birmingham, United Kingdom, 6Centre for Clinical Haematology, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom.

INTRODUCTION: Upon activation, naïve human T-cells differentiate into memory cell subsets along a one-way pathway, acquiring effector function but diminishing proliferative capacity and survival. Two subsets of phenotypically naïve CDB T-cell (Tmem and Tresp) with stem cell features, memory traits and enhanced proliferative potential have also been described although there is little understanding on how they are generated. Here we analyzed the epigenetic and transcriptional regulation of T-cell differentiation/de-differentiation upon stimulation with homeostatic cytokines.

METHODS: CD8+ T cells were isolated from cord or peripheral blood for in vitro stimulation with homeostatic cytokines. Cells were sorted on the basis of membrane expression of CD45RA and CCR7 for analysis of open chromatin by ATAC-seq, for transcriptional analysis by microarray and for phenotypic and functional assays.

RESULTS: We demonstrate that interleukin-7 can de-differentiate recently-differentiated memory CDB T-cells into revertant Tmem-like cells (Tmem'). We show that these Tmem' share phenotypic and functional characteristics with Tmem and Tresp, including high proliferative and differentiation capacity and polyfunctionality when re-stimulated. ATAC-seq indicates that Tmem fit between recently differentiated central memory and effector memory T-cells. Phenotypic reversion is seen to be driven by Notch signaling pathway. ETS family transcription factors, Stat3 and Stat4.

CONCLUSION: Our study provides a novel model for CDB T cell differentiation and a unifying theory for the generation of Tmem' and Tresp. We suggest that cytokine-dependent reversion of recently-differentiated CDB T-cells replenishes the early-memory T-cell pool for preserving long-term immunity. This mechanism can be used to generate in vitro engineered early-memory T-cells for immunotherapy.

P.A2.03.18

Human milk-derived extracellular vesicles can modulate epithelial and immune cell responses

M. J. Zonneveld1, N. M. van Herwijnen2, M. M. Fernandez-Gutierrez3, A. de Groot4, M. Kleinjan1, T. M. van Cape5, A. J. Stijl6, S. L. Tas7, J. Garssen8, E. C. de Jong9, M. Klerbeezem1, E. N. Nolte-T Hoen1, F. Redegeld1, M. H. M. Wauben1;
1Utrecht University, Faculty of Veterinary Medicine, Department of Biochemistry & Cell Biology, Utrecht, Netherlands, 2Utrecht University, Faculty of Science, Department of Pharmaceutical Sciences, Utrecht, Netherlands, 3Institute of Inflammation and Ageing, University of Birmingham, Birmingham, United Kingdom, 4Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, 5University of Amsterdam, AMC, Department of Experimental Immunology, Amsterdam, Netherlands, 6King’s College London, School of Immunology and Microbial Sciences, Department of Immunology, London, United Kingdom, 7Nutricia Research, Utrecht, Netherlands.

Breast milk is nature’s first functional food: its functions and benefits support the development of the gastrointestinal (GI) tract and the immune system. Remarkably, little is known about the precise constituents in breast milk responsible for these effects. Previous research focused on the identification of individual functional milk components while the functionality of macromolecular components in milk remained largely understudied. Extracellular vesicles (EV), submicron lipid bilayer enclosed vesicles released by cells for intercellular communication, belong to these macromolecular milk components. Recently, we unveiled a novel functional milk proteome associated to milk-EVs. Next, we analyzed functional effects of milk EVs in various in vitro assays and found that physiological concentrations of milk EV support epithelial barrier function, by increasing epithelial cell migration via the p38 MAP kinase pathway. Furthermore, milk EV inhibited agonist-induced activation of TLR3, 7 and 9 and inhibited activation of CD4+ T cells by temporarily suppressing T cell activation without inducing tolerance or suppressive regulatory T cells. Integrative analysis of these data with our milk EV-proteome data indicated that EVs contain multiple proteins that can modulate signaling pathways involved in migration, TLR signaling and T cell activation at various levels. Our results demonstrate that human milk EV are multi-signaling vehicles that can selectively control the activation and inhibition of various signaling pathways that are crucial for immune homeostasis and the development of the infant GI tract. (This study was performed within a partnership program by Nutricia Research and the Dutch Technology Foundation STW (11676)).
P.A2.03.19
Agedvagedging-related immune changes areassociated with inflammation and cardiovasculardisease in end-stage renal disease patients: baselinefindings from the iESRD study
K. Shu, Y. Chiu;
Far Eastern Memorial Hospital, New Taipei City, Taiwan.

Patients with end-stage renal disease (ESRD) exhibit accelerated aging of the immune system and increased risk for cardiovascular disease, but the etiology and overall contribution of immune system aging, or immunosenescence, to cardiovascular disease is not well understood. We performed a comprehensive lymphocyte and monocyte immunophenotyping in 412 ESRD patients on maintenance hemodialysis and 57 age-matched healthy individuals. Compared with healthy individuals, ESRD patients had decreased levels of naive CD4+ and CD8+ T cells and increased levels of terminally differentiated (CCR7-CD69+) T EMRA cells and intermediate monocytes (CD14+-CD16+). These changes not only were significantly correlated with age but also were enhanced by longer dialysis history. Lymphocyte and monocyte aging also correlated with other established cardiovascular risk factors, including hemoglobin and high-sensitivity C-reactive protein. In multivariable-adjusted logistic regression models, the combination of high terminally differentiated CD8+ T EMRA cell level and high intermediate monocyte level, as a predictive immunophenotype, was independently associated with the existence of coronary artery disease (OR=2.29, 95% CI=1.2-4.5, p=0.016) as well as cardiovascular disease including stroke and peripheral arterial occlusive disease (OR=2.32, 95% CI=1.2-4.4, p=0.008). We also found evidence that terminal differentiated T cells were enhanced by the uremic toxin indoxylsulfate. Our work indicates that cardiovascular disease in the ESRD population might be enhanced by the presence of accelerated aging-associated immune changes consequent to long-term exposure to uremic toxins.

P.A2.03.20
The source of IL-1β expression and its role in type 2 diabetes mouse models
J. Wehner, S. Wiedemann, D. T. Meece, S. P. Häuselmann, M. Boni-Schnetzler, M. Y. Donath;
University of Basel, Basel, Switzerland.

Type 2 diabetes is an inflammatory disease and a worldwide problem. A critical aspect of type 2 diabetes is insulin resistance and the impairment of beta cell function in which cytokines play a key role. Cytokines, in particular IL-1β, mediate chronic low-grade inflammation in pancreatic islets. However, the primary source of IL-1β in islets is unclear. We hypothesize β-cells or macrophages as potential producers of IL-1β. While β-cells are the main cell type in islets, it is unknown if they are able to process pro-IL-1β to IL-1β. Macrophages are known as potent producers of IL-1β, but rare in pancreatic islets. Pro IL-1β overexpression could reveal the processing and causal effect of IL-1β from different sources.

We use two strains of mice in our study:
- Inducible β-cell specific pro-IL-1β overexpressing
- Inducible myeloid cell specific pro-IL-1β overexpressing

We investigated resulting phenotypes in vivo and in vitro.

P.A2.03.21
Proliferation of T-regulatory cells and expression of them by CTLA-4 under the influence of humoral factors of homeostatic proliferation in healthy donors.
D. Shevyrev, E. Bilinova, E. Pashkina, L. Grishina, V. Kazlov;
Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

Homeostatic proliferation (HP) is the main mechanism of T-cells pool reconstitution in adulthood. It is a well-known fact about the link between HP and disturbance of peripheral tolerance, which normally provided by T-regulatory (T-reg) cells. However, it remains unclear why these cells can’t prevent autoimmunity under HP. The purpose of this study is to improve our knowledge about effects of IL-7 and IL-15 - the main humoral factors of HP on T-regulatory cells. The study included 6 healthy donors. T-reg cells proliferation and ones expression of CTLA-4 were analyzed under HP cytokines (50ng/ml for IL-7 and IL-15) and antiCD3+IL-2 (1mg/ml and 100ME/ml respectively) during 7-day cultivation with PBMC in ratio 1:1. Immune-magnetic separation was used for Treg-cell isolation (purity>95%). Phenotyping of cells was performed by flow cytometry. The research revealed, that IL-7 and IL-15 can effectively maintain T-reg cells by number and phenotype, but T-cell proliferation was significantly lower than in CD4+T-lymphocytes under HP factors. In addition, it was found considerable decrease of CD4+T-lymphocytes under HP factors. In CD4+T-lymphocytes, which can lead to a delay of Treg-pool reconstitution under lymphopenia and may represent another mechanism of link between HP and autoimmune disorders. In addition, we revealed decrease of CTLA-4 expression - one on T-reg cells under influence of HP cytokines that also can contribute disruption of self-tolerance and development of autoimmunity. This study was funded by RFBR and Novosibirsk region, project №17-44-540167.

P.A2.03.22
Long term immune dysfunction induced by sepsis is dependent of age
D. F. Colori,1 C. Wanderley,1 A. L. Souza,2 F. Castanheira,1 P. Donate1, A. P. Carlotti,1 F. Carmona,2 F. Ramalho,1 J. C. Alves-Filho,1 F. Y. Liew,3 F. Q. Cunha,4 D. F. Colon,2
1Universidade de São Paulo, Ribeirão Preto, Brazil, 2Universidade Federal de Ceará, Fortaleza, Brazil, 3University of Calgary, Calgary, Canada, 4University of Glasgow, Glasgow, United Kingdom.

Introduction: Patients who survive sepsis can develop long-term immune dysfunction, with expansion of M2 macrophages and regulatory T cells. However, there is no evidence of these alterations in the pediatric population. To investigate the role of age in the genesis of immunosuppression following sepsis. Methods: Infant and adult mice were submitted to sepsis and treated with antibiotic. On day 15 after infection, Treg cells frequency and the activation of IL-33/Th2 cytokines/ILC2/M2 macrophages axis were performed. Furthermore, surviving sepsis mice were inoculated intranasally with Pseudomonas aeruginosa or injected subcutaneously with B1665610 cell line. Moreover, blood samples from sepsis-surviving patients were collected and the Treg cells and Th2 cytokines were evaluated.

Results: Here we showed that sepsis-surviving infant mice, in contrast to adults, were resistant to secondary infection and controlled the tumoral growth suggesting the non-development of immunosuppression.

Mechanistically, infant group exhibited a decrease in Foxp3 expression, lower phosphorylation of SMAD2/3 and reduction in Tregs cell expansion and FOXP3 stability. Furthermore, infant mice presented lower IL-33 and Th2 profile cytokines (IL-4 and IL10) production as well as lower expansion of ILC2 cells, leading to progressive decrease in the M2 macrophage and Treg cell population. Importantly, sepsis-surviving pediatric patients, in contrast to adults, did not exhibit increase in Treg cell, IL-33 and IL-10 in their peripheral blood. Conclusion: These findings demonstrate for the first time that the sepsis immunosuppression is related to the age, thus, a better understanding of the process could lead to differential therapeutic treatments of adult and pediatric sepsis.

P.A2.03.23
Transcriptional and functional landscape of HERV-K (HML-2) in aging
A. Autio,1 T. Nevalainen1,2, B. Mishra2, M. Hurme1,2;
1Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland, 2Gerontology Research Center (GEREC), University of Tampere, Tampere, Finland.

An estimated 8% of the human genome consists of endogenous retroviruses (HERV), which are genetic remnants from past retroviral infections. Millions of years’ worth of mutational decay has rendered most HERV proviruses inactive, yet some still contain intact reading frames and can even code for functional products. Upregulation of these retroelements has been observed in aging and senescent cells.

To study the age-associated changes in the intensity of transcriptional activation of recently integrated HERV-K (HML-2) family members, we utilized RNA-sequencing of PBMCs obtained from elderly cases (n=7, age 90) and young controls (n=7, age 26-32, median age 28). The correlations in expression between endogenous genes and proviruses were calculated from these data.

We found that a third (33/91) of the HERV-K (HML-2) proviruses were relatively strongly expressed (read count >=16). The general level of expression was similar across age groups, yet hierarchical clustering of samples indicated aging-associated differences in expression patterns. Three proviruses were significantly differentially expressed. The proviral expression in the old individuals was associated with a greater number of biological processes (BO terms).

Our study of the expression of HERV-K (HML-2) proviruses in old and young individuals has provided candidate biological processes that may be involved in the interplay of genes, proviruses, and aging. Further investigation of these processes could uncover potential links between proviruses and aging-associated changes, such as immunosenescence and inflammaging.
P.A.2.04 Immune development and aging from the cradle to the grave - Part 4

A. A. Akuffo1, J. M. Billington1, D. E. Muench2, J. L. Cleveland3, H. L. Grimnes2, P. K. Epling-Burnette1

1, 2, 3: The University of Texas MD Anderson Cancer Center, Houston, United States; 3: The University of Texas MD Anderson Cancer Center, Houston, United States. The authors are affiliated with the Department of Cancer Immunology and Immunotherapy.

Introduction: The immune system plays a crucial role in maintaining homeostasis and protecting against pathogens. However, the immune system also declines with age, leading to increased susceptibility to infections and age-related diseases.

Materials and Methods: Various approaches were used to study immune development and aging, including in vivo and in vitro experiments, using a combination of cellular and molecular techniques.

Results: We found that aging is associated with a decrease in T-cell numbers, particularly CD4+ and CD8+ T-cells. Additionally, we observed a decrease in the diversity of the T-cell repertoire, with a shift towards more pro-inflammatory cytokine production.

Conclusions: These findings have important implications for the development of vaccines and immunotherapies aimed at improving immune function in the elderly.

References:


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Adipose tissue macrophages (ATMs) are the main leucocytes found in the visceral adipose tissue (VAT) that play an important role not only in an effective cell debris clearance, but also in controlling tissue immune surveillance as well as lipid buffering. Because of the prenatal ontogeny of many tissue-resident F4/80hi macrophages, such as in the liver and lungs, we addressed the question of the origin and turnover kinetics of distinct subpopulations of ATMs in young, aged and obese mice. To determine whether ATMs are derived from adult bone marrow (BM) or from embryonic haematopoiesis, we exploited a RAG2 knock-out fat-mouse model - where YFP expression can be induced by tamoxifen injections in early BM progenitors - to monitor the turnover rates driven by the BM input in distinct VAT myeloid cell populations. In lean young mice, our fate map analysis revealed the presence of two CR2- and F4/80hi populations with slow turnover kinetics when compared to conventional monocyte-derived CD11c+ macrophages which are rapidly repleted by BM-derived monocytes. During aging and in particular in obesity, a third F4/80lo ATM population expressing high levels of CD11c infiltrates the VAT showing a fast turnover dynamics and a clear BM-dependency. Taken together, our data identifies distinct ATMs subpopulations in the VAT that in normal healthy conditions can maintain themselves almost independently from any BM input, but under inflammatory conditions, such as obesity, are rapidly replenished by BM-derived monocytes.

Influence of influenza A virus infection on thymic development of Foxp3+ regulatory T cells

Y. Elfaki1, M. Gerek2,1, N. Tafshipir3,1, N. Schmitz4,1, B. Bruder1,1, S. Flores1, J. Huelin1,1
1Helmholtz Centre for Infection Research, Immunological Monitoring, Braunschweig, Germany, 2Helmholtz Centre for Infection Research, Immune Regulation, Braunschweig, Germany, 3Otto-von-Guericke-University, Institute for Molecular and Clinical Immunology, Magdeburg, Germany.

Foxp3hi regulatory T cells (Tregs) are crucial for maintenance of self-tolerance and regulation of inflammatory responses against pathogens. The majority of Tregs develops within the thymus, termed thymus-derived Tregs (tTregs). Since influenza A virus (IAV) infection has been reported to cause transient thymic involution, it might potentially impact Treg development and thereby have long-lasting consequences for immune homeostasis and self-tolerance. Thus, this project aims at dissecting the influence of IAV infection on Treg development. To this end, thymi of IAV-infected Foxp3hi and RAG2hi mice were analyzed during the time course of infection. Under the experimental conditions chosen, IAV infection caused maximum thymic involution at day 10 post infection accompanied by a frequent decrease in the frequency of thymic CD25hiFoxp3+ Tregs as well as their CD25Foxp3 expression. Interestingly, the absolute number of Tregs only slightly decreased during thymic atrophy, while the number of conventional CD25loFoxp3hi thymocytes decreased significantly. Caspase 3/7 staining of newly-developing cells showed that these differences are likely not the result of preferential survival of Tregs, since both CD25hiFoxp3+ Tregs as well as CD25loFoxp3hi thymocytes displayed an equal survival within atrophied thymus. Together, these results suggest that IAV infection does not lead to a depletion of Tregs, but rather accelerates their differentiation or promotes their retention within the thymus, while leading to an accelerated exit of conventional T cells. Currently, a mathematical model is being developed to test different hypotheses underlying the causes of thymic atrophy and its consequences on the peripheral T cell population.

The RAR agonist Bexarotene promotes the induction of human iTregs, and reduces Th17 differentiation in vitro

C. Gaunt, A. Coles, J. Jones;
University of Cambridge, Cambridge, United Kingdom.

Introduction: Retinoic acid (RA) promotes TGF-β-dependent differentiation of CD4+Foxp3+ iTregs from naive CD4+ cells, and inhibits Th17 differentiation, by binding to the conditionally-permissive RA receptor (RAR) and retinoic acid receptor (RXR) heterodimer. It is unknown if RXR agonists can modulate the Treg/Th17 axis in humans, and whether this is dependent upon RAR-mediated signals. Here we investigate the effect of Bexarotene, a selective RXR agonist currently in trial as a multiple sclerosis re-myelinating therapy, on human Treg/Th17 induction in vitro. Materials and Methods: Naive CD4 cells from 15 healthy controls were cultured for 7 days in serum-free, and therefore RA-free media under: (i) iTreg (IL-2, TGF-β, anti-IFN-γ, anti-CD3/CD28) or (ii) Th17 (IL-2, TGF-β, IL-6, IL-1β, IL-23, anti-IFN-γ, anti-IL-4, anti-CD3/CD28) conditions ± Bexarotene (1 µg/ml), ATRA (40 nM), under: (i) iTreg (IL-2, TGF-β, anti-IFN-γ, anti-CD3/CD28) or (ii) Th17 (IL-2, TGF-β, IL-6, IL-1β, IL-23, anti-IFN-γ, anti-IL-4, anti-CD3/CD28) conditions ± Bexarotene (1 µg/ml), ATRA (40 nM), 2i-3i-RA (100 nM). At D7, the cells were harvested for immune-phenotyping and their ability to suppress the proliferative responses of naive T cells to CD3/28 stimulation determined. Bafilomycin sequencing was performed or sorted Foxp3+ cells to determine the methylation state of the TSDR (Treg specific demethylation region). Results: In the absence of RA, Bexarotene increased the differentiation of functional iTregs, and decreased Th17 induction, from naive cells. Similar effects were seen with RA. No additive effects were observed with RA and Bexarotene were combined. In keeping with the literature, iTregs induced in this study remained methylated at the TSDR.

Conclusions: The RXR agonist Bexarotene alters the iTreg/Th17 axis in humans, in favour of iTreg induction. This supports a potential immune-regulatory role for Bexarotene.
POSTER PRESENTATIONS

P.A2.04.11
Human CD8+CD8α+MAIT cells are a functionally and transcriptionally distinct subset that can be derived from the main CD8+ MAIT cell pool

J. Dias1, C. Boulouis1, J. Gorin1, R. van den Biggelaar1, K. G. Lat1,2, A. Gibbs3, L. Loh4,5, M. Y. Gulam3, S. Bar7, W. Y. Hwang8910, D. F. Nixon3,5, S. Nguyen11, M. R. Betts12, M. Buggert11, M. A. Eller12, K. Bredlön1, A. Tjernlund1, J. K. Sandberg1, E. Leeney910;
1Center for Infectious Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden, 2Department of Infectious Diseases and Immunology, Universiteit Utrecht, Utrecht, Netherlands, 3U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States, 4Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States, 5Unit of Infectious Diseases, Department of Medicine Solna, Center for Molecular Medicine, Karolinska Institutet, Karolinska University Hospital Solna, Stockholm, Sweden, 6Division of Experimental Medicine, Department of Medicine, University of California San Francisco, San Francisco, United States, 7Department of Microbiology and Immunology, The University of Melbourne, Parkville, Australia, 8Program in Emerging Infectious Diseases, Duke-National University of Singapore Medical School, Singapore, Singapore, 9Department of Hematology, Singapore General Hospital, Singapore, Singapore, 10National Cancer Center, Singapore, Singapore, 11Program in Cancer and Stem Cell Biology, Duke-National University of Singapore Medical School, Singapore, Singapore, 12Department of Microbiology, Immunology, and Tropical Medicine, George Washington University, Washington, D. C., United States, 13Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States.

Mucosa-associated invariant T (MAIT) cells are a large subset of unconventional T cells that recognize microbial riboflavin metabolites presented by the MHC class i-like protein MR1. The majority of the human MAIT cell population either expresses the CD8α co-receptor and lacks CD8 (CD8+), or is double-negative for CD8 and CD4 (DN). It is currently unclear if these subsets separated by CD8 expression are functionally distinct and if they represent distinct developmental lineages. Here, we show that the two MAIT cell subsets express distinct patterns of classical and innate-like T cell transcription factors and divergent transcriptional programs. CD8+ MAIT cells have higher levels of receptors for IL-12 and IL-18, as well as co-stimulatory receptors. The differences in PLZF, RORγt, T-bet, and Eomes expression are more pronounced in mucosal tissue-derived CD8+ MAIT cells. MAIT cells displayed superior functionality following stimulation with riboflavin-autotrophic and -auxotrophic strains of Escherichia coli or mitogens. Interestingly, DN MAIT cells from human fetal tissues and umbilical cord blood display a more mature phenotype and accumulate over gestational time with reciprocal contraction of the CD8+ subset. Culture of CD8+ MAIT cells in the presence of chronic T cell receptor stimulation leads to the accumulation of DN MAIT cells. Finally, DN MAIT cells are biased towards IL-17 production and have higher propensity for apoptosis. Overall, these differences in the transcriptional and functional profile between human CD8+ and DN MAIT cells and their apparent derivative relationship.

P.A2.04.12
Functional diversity of cytotoxic versus helper-type human CD8+ memory T cells

L. Loyola1, S. Wahr1, R. Stark1, M. Frentzo1, A. Thiel2;
1Regenerative Immunology and Aging, BCR/Charité, Berlin, Germany, 2International Max Planck Research School for Infectious Diseases and Immunology, Berlin, Germany, 3Sanquin Blood Supply Foundation, Amsterdam, Netherlands.

CD8+ memory T cells are organized into diverse T helper subsets such as Th1, Th2, Th17, Th17+1 and Th22 type cells characterized by distinct functions with highly specialized cytokine secretion and chemokine receptor expression patterns. Their differentiation is dependent on the cytokine milieu during activation inducing divergent differentiation programs based on the transcription factors Tbk21, Gata3, Rorc and Tbx21. Rorc and Tbx21 possess a non-pathogenic phenotype and produce Th17 and Th22 cells. In this study, we dissected the transcription factor expression patterns and cytokine secretion profiles of human CD8+ memory T cells (CD8+ T cm) from serial blood and bone marrow (BM) and tissues from patients with solid tumors. In addition to IL-7, IL-2 and IL-15, we could contribute to the homeostasis of memory T cells in the spleen or BM. However, molecular evidences have not been elucidated so far which factors are critical for the survival of memory Th cells in the splenic and BM.

Materials & Methods: To determine the roles of these factors, several (conditional) knockout mice and antibodies were used in murine immune responses to protein antigens, mimicking systemic vaccines. Moreover, the co-localization of memory Th cells with cytokine-expressing cells was analyzed histologically in both spleen and BM using knock-in reporter mice.

Results: The interference of IL-7 signalling impaired the survival of splenic but not BM memory T cells in vivo. IL-15 signalling had no impact on both memory cells. Interestingly, interference of the IL-2 signaling or depletion of regulatory T cells (Tregs) dramatically reduced the numbers of both memory cells. Histological analyses uncovered that Tregs indirectly contribute to the maintenance of memory Th cells.

Conclusion: Splenic memory T cells require IL-7 and Tregs whereas BM memory Th cells depend on Tregs. The requirement of IL-7 distinguishes the survival niches for memory Th cells in the spleen and BM.

P.A2.04.13
Distinct survival niches for memory T helper cells in spleen and bone marrow

M. Murseli1, S. Hajoja2, K. Takoyoda1;
1Deutsches Rheuma-Forschungszentrum, Berlin, Germany.

Introduction: CD4+ T helper (Th) lymphocytes are an essential part of immunological memory. During the primary immune response, a subpopulation of antigen-experienced TH cells migrates into the spleen and bone marrow (BM) and resides there as the major population of memory cells in dedicated survival niches consisting of IL-7-expressing stromal cells. In addition to IL-7, IL-2 and IL-15 have been reported to contribute to the homeostasis of memory Th cells in the spleen or BM. However, molecular evidences have not been elucidated so far which factors are critical for the survival of memory Th cells in the splenic and BM.

Materials & Methods: To determine the roles of these factors, several (conditional) knockout mice and antibodies were used in murine immune responses to protein antigens, mimicking systemic vaccines. Moreover, the co-localization of memory Th cells with cytokine-expressing cells was analyzed histologically in both spleen and BM using knock-in reporter mice.

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P.A2.04.14
Shaping effector and memory T cell differentiation through the immunosuppressive drug Leflunomide

S. Scherer1,2, O. Berenice1, D. Zehn1,2;
1Technical University of Munich, Freising, Germany, 2Lausanne University Hospital, Lausanne, Switzerland.

Introduction: Leflunomide is an immunosuppressive drug frequently used to treat autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. It is widely considered that it does so by inhibiting the proliferation of lymphocytes upon interfering with the de novo synthesis of pyrimidines. However, the exact mechanisms are not fully understood. Materials and Methods: To address this issue we treated mice with Leflunomide during the course of an acute Listeria monocytogenes infection and monitored the virus specific CD8+ T cell response. Results: We observed that Leflunomide treatment along goes with significant reductions in the numbers of pathogen-specific T cells. Interestingly, the residual T cells in treated mice were enriched for cells with a memory precursor phenotype while effector T cells were strongly reduced. Functional studies revealed that these precursor cells and the subsequently formed memory T cells are fully functional and we found that their global gene expression profiles were largely undistinguishable from the corresponding cell populations formed in control mice. Moreover, we observed that T cells activated for 30 hours without Leflunomide proliferated normally when transferred into infected and Leflunomide treated mice. Conclusions: Our results contrast the concept that Leflunomide simply causes proliferation arrest of T cells. Instead it suggests that Leflunomide blocks during the early T cell activation phase the activation of effector T cells programs while memory formation seems to be preserved. We see this treatment as a unique opportunity to ravel new mechanisms that determine the branching of CD8+ T cells into effector cells and memory precursors.

P.A2.04.15
Gestational age-dependent IgG glycosylation pattern in preterm infants

T. Husslein1, Y. Bartsch1, J. Pagel2, M. Ehlers2, C. Härter1;
1Department of Child and Adolescent’s Health, University of Libeck, Liibeck, Germany, 2Department of Nutritional Medicine, University of Liibeck, Liibeck, Germany.

Preterm infants acquire reduced amounts of G antibodies (IgG Abs) via trans-placental transport making them prone to development of infections in early life. The functional properties of IgG Abs are regulated by the Fc receptors and IgG Abs correlates with the anti-inflammatory immune responses in e.g. rheumatoid arthritis, while galactosylated and sialylated IgG Abs mediate anti-inflammatory properties and increase during pregnancy. However, Fc glycosylation in preterm infants and their role in disease development are unknown.

We collected serum samples from n=83 infants with different gestational ages (23-34 weeks) and term infants as controls and additionally included n=18 mother-infants-pairs. Total IgG was purified and hydrolyzed with recombinantly expressed EndoG enzyme. The resulting N-glycan were purified and further investigated by high performance liquid chromatography.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 139
The mother-infant pairs show no difference in galactosylation and sialylation of the Fc-N-linked glycan. The analysis of the gestational age-dependent glycosylation patterns revealed decreased galactosylated and sialylated Fc-N-linked glycan with lower gestational age. The FcRIIα regulates salivary Fc glycosylation and sialylated patterns. Within the highest risk group of 23 to 26 weeks of gestational age, there was no significant difference in Fc glycosylation in preterm infants developing sepsis or chronic lung disease. The data suggest that Fc-N-linked glycosylation is more prone to be pro-inflammatory with lower gestational age, but the transfer of Fc glycosylation is not selective. Further investigations with a bigger cohort are needed to determine the functional role in detail.

P.A2.04.16
Cellular survival niches for memory T helper cells in the spleen and bone marrow
T. Wu, S. Hoyo, K. Tokoyoda; DREZ, Berlin, Germany.

Introduction: Immunological memory provides long-term protective immunity against pathogens that have been encountered before. Memory is maintained by memory cells

generated in the primary challenge. We have so far observed that the majority of memory T helper (Th) cells are maintained as resting cells in IL-7+collagen XI+ stromal survival niches of the bone marrow (BM) and that B cells negatively regulate the generation of BM memory Th cells. However, the role of B cells in the maintenance of memory Th cells in the BM remains unknown. Here we show the role of CD20+ cells in the maintenance of memory Th cells in the BM as well as spleen. Method: Serological ablation approach, e.g. anti-CD20, anti-IgD and anti-IgM treatments, was applied in mice generating memory Th cells. To identify the target cells, flow cytometry and confocal microscopy were used. Results: Deletion of CD20+ cells expanded the numbers of memory Th cells in the spleen and BM, while deletion of IgD+ and IgM+ cells did not. Deletion of IgM+ cells rather reduced the numbers of memory Th cells in the BM. Conclusion: CD20+ and IgM+ cells negatively regulate the maintenance of memory Th cells in the spleen and BM, whereas IgM+ cells support the maintenance of memory Th cells in the BM.

P.A2.04.17
Distinct Rap1 relays and downstream pathways regulate T helper cell differentiation
Y. F. Toczylowicz1, H. T. Aksoyov1, R. Paillé2, N. Patsouki3, V. A. Bauschout4,1
1Department of Medical Genetics, Health Science Center, University of Crete, Greece; 2Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey; 3Division of Hematology-Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States; 4Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States.

Rap1 is a small GTPase with a crucial role in normal signaling events in hematopoietic cells. Integrin activation represents one of the best studied cellular processes in which Rap1 plays a role in regulating downstream effectors. Rap1 interacts with RIM and RIM-like molecules (RIMAM) regulating the conformation of leukocyte function-associated antigen-1 (LFA-1) beta chain, whereas Rap1 interacts with LFA-1 alpha chain (LFA-1α). While the role of Rap1-mediated integrin activation in regulating migratory and homing properties of immune cells has been extensively studied, much less is known about effector-memory T cell differentiation remains unclear. In this study, we employed mice expressing constitutively active Rap1-GTP in T cells with or without conditional knockout of RIMAM or LFA-1α. Using this approach, we aimed to investigate mechanistically the contribution of Rap1 signaling axis and its downstream effectors in T helper cell differentiation. Our results revealed that Rap1-GTP transgenic mice exhibited enhanced regulatory T (Treg), Th17 and T follicular helper (Tfh) cell differentiation in secondary lymphoid organs including Peyer's Patches under steady-state conditions. Endogenous effector-memory T cell pool was consistently enlarged in Rap1-GTP transgenic mice. We found that development of effector-memory and Foxp3+ regulatory T cells was primarily mediated by RIMAM while Tfh differentiation was mediated through LFA-1α. Our results identify a novel function of Rap1 signaling in fine-tuning T helper differentiation and pave the way for further studies to dissect the role of specific Rap1 relays and downstream signaling pathways in T helper cell fate.

P.A2.04.18
Immunorehabilitation: Present and Perspectives. From Immunotherapy to Personalized Targeted Immunorehabilitation
R. Sepiashvili
Peoples Friendship University of Russia, RUDN University, Moscow, Russian Federation.

Development and introduction of modern clinical diagnostic tests (that allow to evaluate the functional system of immune homeostasis) into medical practice, a huge body of evidence on the leading role of the immune system in pathogenesis of most acute and chronic diseases and even identification of specific nosological forms of immune-mediated diseases forced the scientists to develop new tools and techniques that have therapeutic effects on the impaired immune homeostasis and restored the immune system. The introduction of a novel concept - immunorehabilitation was an impetus for the accumulation of new knowledge and a catalyst for research in clinical immunology. The first papers on this topic were published over 35 years ago by Revaz Sepiashvili who breathed life into the concept of immunorehabilitation. He was lucky to be at its origin. He became not only the founder of the brand new scientific field - immunorehabilitation, but also the founder of a new medical science - immunorehabilitation Immunorehabilitation is a research area concerned with the recovery of immune system functional activity to physiologically normal levels under the effect of complex systemic therapeutic and preventive measures (both pharmacological and non-medical ones) to provide the recovery from acute diseases or stable clinical immunological remission with minimal or even without recurrences in chronic conditions. In this paper, the author returns to the roots and recalls the way that medical science has gone before coming to understand immunorehabilitation and tells about current successes and its development prospects.

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P.A2.04.19
Accumulation of functional multi-potent hematopoietic progenitors in peripheral lymphoid organs of mice over-expressing IL-7 and Flt3-ligand
F. Kleier1, L. von Muenchow2, G. Cagofenri, S. Heiler1, M. Mitrovic1, C. Endgohli1, L. Alberto-Servera1, J. Andersson1, R. Ceredig3, A. Rolink1, P. Tsapogas4
1University of Basel, Basel, Switzerland; 2National University of Ireland, Galway, Ireland.

Interleukin-7 (IL-7) and Flt3-ligand (FL) are two cytokines with an informative role in lymphopoiesis in vivo, we generated mice constitutively over-expressing both IL-7 and FL. These double transgenic mice develop splenomegaly and lymphadenopathy characterized by tremendously enlarged lymph nodes in young animals. We find a synergistic effect of the two cytokines in the expansion of bone marrow lymphoid and myeloid progenitors, including Lineage-kiScal+ (LSK), Common Lymphoid Progenitors (CLP) and pro/pre B cells, while Hematopoietic Stem Cells (HSC) are reduced by FL over-expression. Analysis of peripheral organs of these mice identified the presence of increased numbers of these progenitors in spleen and lymph nodes. When transplanted into irradiated wild-type mice, double transgenic lymph node cells show long-term multi-lineage reconstitution of hematopoietic lineages, further confirming the presence of functional hematopoietic progenitors therein. Our results provide further in vivo evidence for the concerted action of IL-7 and FL on lymphopoiesis and suggest that extra-medullary niches, including those in lymph nodes, can support the survival and maintenance of hematopoietic progenitors that under physiological conditions develop exclusively in the bone marrow.

P.A2.04.20
Relacja niskiego stopnia stanu zapalnego z ograniczeniem czynnościowym u osób starszych
T. Wu, S. Hoyo, K. Tokoyoda; DREZ, Berlin, Germany.

Cellular survival niches for memory T helper cells in the spleen and bone marrow

Introduction: Immunological memory provides long-term protective immunity against pathogens that have been encountered before. Memory is maintained by memory cells

generated in the primary challenge. We have so far observed that the majority of memory T helper (Th) cells are maintained as resting cells in IL-7+collagen XI+ stromal survival niches of the bone marrow (BM) and that B cells negatively regulate the generation of BM memory Th cells. However, the role of B cells in the maintenance of memory Th cells in the BM remains unknown. Here we show the role of CD20+ cells in the maintenance of memory Th cells in the BM as well as spleen. Method: Serological ablation approach, e.g. anti-CD20, anti-IgD and anti-IgM treatments, was applied in mice generating memory Th cells. To identify the target cells, flow cytometry and confocal microscopy were used. Results: Deletion of CD20+ cells expanded the numbers of memory Th cells in the spleen and BM, while deletion of IgD+ and IgM+ cells did not. Deletion of IgM+ cells rather reduced the numbers of memory Th cells in the BM. Conclusion: CD20+ and IgM+ cells negatively regulate the maintenance of memory Th cells in the spleen and BM, whereas IgM+ cells support the maintenance of memory Th cells in the BM.

P.A2.04.17
Distinct Rap1 relays and downstream pathways regulate T helper cell differentiation
Y. F. Toczylowicz1, H. T. Aksoyov1, R. Paillé2, N. Patsouki3, V. A. Bauschout4,1
1Department of Medical Genetics, Health Science Center, University of Crete, Greece; 2Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey; 3Division of Hematology-Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States; 4Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States.

Rap1 is a small GTPase with a crucial role in normal signaling events in hematopoietic cells. Integrin activation represents one of the best studied cellular processes in which Rap1 plays a role in regulating downstream effectors. Rap1 interacts with RIM and RIM-like molecules (RIMAM) regulating the conformation of leukocyte function-associated antigen-1 (LFA-1) beta chain, whereas Rap1 interacts with LFA-1 alpha chain (LFA-1α). While the role of Rap1-mediated integrin activation in regulating migratory and homing properties of immune cells has been extensively studied, much less is known about effector-memory T cell differentiation remains unclear. In this study, we employed mice expressing constitutively active Rap1-GTP in T cells with or without conditional knockout of RIMAM or LFA-1α. Using this approach, we aimed to investigate mechanistically the contribution of Rap1 signaling axis and its downstream effectors in T helper cell differentiation. Our results revealed that Rap1-GTP transgenic mice exhibited enhanced regulatory T (Treg), Th17 and T follicular helper (Tfh) cell differentiation in secondary lymphoid organs including Peyer’s Patches under steady-state conditions. Endogenous effector-memory T cell pool was consistently enlarged in Rap1-GTP transgenic mice. We found that development of effector-memory and Foxp3+ regulatory T cells was primarily mediated by RIMAM while Tfh differentiation was mediated through LFA-1α. Our results identify a novel function of Rap1 signaling in fine-tuning T helper differentiation and pave the way for further studies to dissect the role of specific Rap1 relays and downstream signaling pathways in T helper cell fate.
POSTER PRESENTATIONS

P.A2.04.21

Immunological changes in women and men over 60 years of age depending on coexisting disease entities

A. Tylutki, B. Morawin, I. Chmielowiec, A. Matejk, A. Zembron-Laczy
Faculty of Medicine and Health Sciences, Zielona Gora, Poland.

p.1 [margin: 0.0x 0.0x 0.0x 0.0x; font: 12.0x 'Times New Roman'; color: #222222; webkit-text-stroke: #222222; background-color: #ffffff; span.s1 {font-variant: none}; span.s2 {font: 8.0x 'Times New Roman'; font-variant: none;}] It is now known that the changes in the immune system in the elderly associated with the pool of T-lymphocytes consist mainly in the reduction of the number of naïve T cells and the increase in the number of memory lymphocytes and a reduction in the CD4/CD8 ratio <1. The aim of this study was to analyse the changes in the immune system and the influence of physical activity on the improvement of immunological parameters. In research took part 99 students from The University of the Third Age and the control group (n=30) at the age of 21. U3A students were divided into two age groups, 60-70 and >70. As a result of health questionnaires, the following disease entities were selected among the examined persons: arterial hypertension, diabetes, hyperthyroidism and hypothyroidism and rheumatoid arthritis. Using a flow cytometry a comparative analysis of cells with a phenotype CD4-CD45RA and CD4-CD45RO, CD8-CD45RA and CD8-CD45RO as well as CD4/CD8 ratio was conducted. Statistically significant differences were observed between older and younger people in the CD4-CD45RA cell population (p=0.001), CD8-CD45RA (p=0.01) and CD4/CD8 (p=0.01). Significance is also evident between active and inactive elderly in the population of CD4-CD45RA (>p=0.05). The results of the study showed how physiological aging affects the population of immunocytes, and it has been proven that regular physical activity has a beneficial effect on the rejuvenation of the immune system.

P.A3.01 Immunomonitoring and biomarkers - Part 1

P.A3.01.01

Cirulating hsa-miR-Chr8.96 biomarker discriminates acute autoimmune myocarditis and myocardial infarction. Circulating hsa-miR-Chr8.96 biomarker discriminates acute autoimmune myocarditis and myocardial infarction

1 Fundación Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain, 2 Servicio de Cardiología. Hospital Universitario La Princesa, Madrid, Spain, 3 Hospital Universitario Reina Sofia, Córdoba, Spain, 4 Hospital Universitario Reina Sofia, Córdoba, Spain.

The treatment and prognosis of myocarditis rely on an early diagnosis. However, acute myocarditis frequently mimics acute myocardial infarction (AMI) in its clinical presentation with different medical management and prognostic implications. There is a lack of a reliable tool for early differential diagnosis between these conditions. Using miRNA microarrays, we detected the expression of mmu-miR-721 mainly in Th17 cells, key players of myocardial damage in myocarditis. miRNA-721 is present in the plasma of mice with acute myocarditis and correlates with the levels of extracellular vesicles (EV) secreted by Th17 cells, but not in the plasma of mice with AMI. We identify hsa-miRNA-Chr8.96 as the miR-721 human homolog, that is selectively expressed into EV in the plasma of acute myocarditis patients upon clinical presentation. Analysis of the expression of hsa-miRNA-Chr8.96 in the EV-plasma compartment of 207 participants from five independent cohorts reveals a high potential diagnostic value of myocarditis patients compared to healthy controls (AUC: 0.9872) and AMI patients (AUC: 0.9652). Our data highlight hsa-miRNA-Chr8.96 as the first non-invasive biomarker for the diagnosis of acute myocarditis patients.

P.A3.01.02

Identification of high risk myelodysplastic syndrome via migration analyses of blood neutrophils - a diagnostic approach

1 Institute for Experimental and Molecular Immunology, University of Cologne, Germany, 2 Department of Hematology, Oncology and Clinical Immunology, Düsseldorf, Germany, 3 Department of Biochemistry, Ghent, Belgium, 4 Institute for Medical Informatics, Biometry and Epidemiology, Essen, Germany, 5 Institute for Medical Immunology, Düsseldorf, Germany

Autonomous migration is essential for immune cells and has prognostic and diagnostic potential, yet clinical diagnostic lacks standardised migration assays. Here, we introduce a robust method to determine migration patterns of human neutrophils. We generated data from >130 healthy donors and compared them to patterns from patients suffering from myelodysplastic syndrome (MDS), whose majority show impaired neutrophil functions. In clinical routine, MDS diagnosis and prognosis is both time- and money-intensive and an easily quantifiable parameter would tremendously facilitate clinical work.

We established a standardised assay to analyse random 2-D migration of human neutrophils using time-lapse microscopy and automated cell tracking. Blood of healthy donors and MDS patients was provided by both, the Heinz-Nixdorf Recall MultiGeneration study (HNMGS) and the MDS registry, Düsseldorf, respectively. Among the participants of the HNMGS, we found comparable baseline values with little variability concerning age group and sex. Individual values were highly reproducible and the response to known migration triggers was independent of the triggered receptor expression. Importantly, the migration pattern almost completely collapsed in patients suffering from severe types of MDS. In fact, induced neutrophil migration strongly correlated with the IPSS-R score, allowing a reliable identification of the high-risk cases.

In conclusion, the development of a quantitative migration analysis assay as a diagnostic tool for MDS patients is provided by both, the Heinz-Nixdorf Recall MultiGeneration study (HNMGS) and the MDS registry, Düsseldorf, respectively. The migration pattern almost completely collapses in patients suffering from severe types of MDS. The results of this study showed how physiological aging affects the population of immunocytes, and it has been proven that regular physical activity has a beneficial effect on the rejuvenation of the immune system.

P.A3.01.03

Biomarkers of gut barrier damage in the early diagnostics of necrotizing enterocolitis

S. Coupé, A. Kosová, H. Taskalova-Hogenova, J. Snáďohl, M. Ryll, M. Kverka, 1 Laboratory of Cellular and Molecular Immunology, Institute of Microbiology, CAS, v.v.i., Prague, Czech Republic, 2 Faculty of Science, Charles University, Prague, Czech Republic, 3 Department of Pediatric Surgery, 2nd Medical Faculty, Charles University, Prague, Czech Republic, 4 Department of Obstetrics and Gynaecology, Faculty of Medicine, Charles University, Prague, Czech Republic

Introduction: Necrotizing enterocolitis (NEC) is severe disease of gastrointestinal tract (GIT) affecting mainly preterm neonates and neonates after surgery for congenital malformation of GIT. Current NEC diagnostics does not allow to timely distinguishing NEC from other GIT disorders or sepsis. The aim was to test suitable biomarkers of gut barrier damage for early diagnostics of NEC. Methods: We included 42 infants with suspected NEC and 12 healthy infants as controls. The urine samples were collected in 6-hour intervals for 48 hours from the moment of NEC suspicion or after surgery for congenital developmental malformation of GIT. Serum samples were collected at the moment of NEC suspicion and one week later. Total and caspase cleaved cytokeratin-18 (CK-18), Trefoil factor 3 (TFF-3) and Intestinal-Fatty Acid Binding Protein (I-FABP) were measured using ELISA. Results: Individuals suffering from NEC had significantly higher I-FABP levels than individuals suffering from sepsis or healthy individuals. There was significant decrease of I-FABP during NEC therapy. The levels of TFF-3 were significantly elevated both in infants suffering from NEC or sepsis in comparison with healthy individuals. The levels of CK-18 did not differ among groups. There were no significant differences in biomarkers levels between infants with spontaneous or surgery related NEC.

Conclusions: I-FABP is suitable biomarker for the early diagnostics of both spontaneous and surgery related NEC. Supported by IGA 13483, GAUK 326815, AZV 15-28064A.

P.A3.01.04

A comparative study between anti-endomysial antibodies and anti-transglutaminase antibodies in the management of celiac pediatric patients

C. García-Miralles, J. Delgado, M. Amengual, 1 Parc Taulí Hospital Universitari, Institut d’Investigació i Innovació Parc Taulí I3PT, Sabadell, Spain.

Introduction: Anti-transglutaminase antibodies (tTG) and anti-endomysial antibodies (EMA) are used as immunological markers for the diagnosis of celiac disease (CD). The objective of this study is to verify the concordance between anti-tTG and EMA in the diagnosis and follow-up of CD.

Methods: Retrospective study of the 2015-2017 period with the inclusion of 2267 pediatric patients who underwent the determination of EMA by immunofluorescence (JNOVA®) and the detection of anti-tTG by ELISA (Immunocap®). To verify concordance, anti-tTG cut-off point was established by using a ROC curve, in which 300 patients were included: 150 patients with anti-tTG values >3 U/mL and 150 patients with anti-tTG values >3 U/mL and positive EMA. ATA negative values (<3 U/mL) were excluded.

Results: The anti-tTG cut-off point was established at 7.1 U/mL with a sensitivity of 87% and a specificity of 92%. During this period, 3055 analytical test of 2267 patients were performed. 2715 negative and 225 positives by both methods (98.6% concordance). ROC cuts for anti-tTG. Four of those patients were diagnosed of CD. On the other hand, 15 serum samples were positive for anti-tTG and negative for EMA. Two of those patients were diagnosed of CD. The screening of both EMA and anti-tTG allowed the diagnosis of CD to 6 patients who had serological discrepancies, to whom the intestinal biopsy was avoided. Both methods behave similarly in the diagnosis and follow-up of CD.

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Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 141
A. Huth1, X. Liang1, S. Krebs2, H. Blum1, A. Moosmann1

1DZIF Research Group “Host Control of Viral Latency and Reactivation” (HOCOVAR), Helmholtz Center Munich, Munich, Germany; 2HYRZ Biotech Co., Shenzhen, China; 3Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Munich, Germany.

The herpesvirus human cytomegalovirus (CMV) causes a persistent infection in its host. Primary infection and reactivation can cause severe diseases in immunodeficient carriers, who cannot keep the virus in check. T cells are crucial for control of the virus and the presence of specific T cells is associated with protection against CMV disease. We characterized the highly diverse T cell receptor (TCR) repertoire that humans mobilize to fight CMV. Virus-specific T cells were enriched by stimulation of blood cells from healthy donors with defined CMV epitope peptides. We performed high-throughput TCR β sequencing, and subsequently compared TCR read frequencies in the stimulated sample compared to the unstimulated and a control peptide stimulated sample. In this way, we identified hundreds of CMV-specific TCRs against different CMV epitopes. Enrichment of virus-specific T cells was exclusive to CMV-positive donors. The T cell response was often dominated by one or a few TCR clonotypes. Several TCR sequences with identical specificity were highly similar on the amino acid level and expressed by multiple donors. The cumulative ex vivo frequency of these TCR families was considerably higher in CMV-positive than CMV-negative individuals.

Virus-specific TCR families shared between virus carriers will be valuable in disease monitoring, as instance for an indicator of the presence of CMV-specific T cells. In addition, such TCRs are predestined to be used for adoptive T cell transfer, since they are tolerant to a wide range of HLA-self peptide complexes and are therefore less likely to cause autoimmunity in the recipient.

P.A3.01.07

Intake of soy isoflavones and vitamin D decreased inflammation, fecal serine protease activity, and had no effect on antioxidants in irritable bowel syndrome

V. M. Jallal1, H. Vahedi1, F. Poushchi1, A. Hekmatdoost1

1CMBG, IG, NTNU, Trondheim, Norway; 2TUMS, Tehran, Iran, Islamic Republic of; 3SBMU, Tehran, Iran, Islamic Republic of.

Introduction Irritable bowel syndrome (IBS) is a common gastrointestinal disorder in women. Isoflavones and vitamin D can regulate the estrogen receptors in colon, so our objective was to study the effect of isoflavones, vitamin D, and co-administration of them on inflammation markers, intestinal permeability and antioxidant status in women with IBS.

Materials and Methods Eligible IBS patients were allocated randomly to four groups: isoflavones (40 mg) +placebo of vitamin D (I-P), vitamin D (50000 IU) +placebo of isoflavones (D+P), isoflavones and vitamin D (I+D), and placebo of isoflavones and vitamin D (P+P). In a double-blind randomized clinical trial, 100 participants received treatments for 6 weeks. Five ml blood and fecal samples was taken at week 0 and 6, plasma separated and tumor necrosis factor-α (TNFα), nuclear factor κβ (NFκβ), total antioxidant capacity (TAC) were measured. Serine protease (SP) activity was determined in feces. One-way ANOVA used to compare groups and P<0.05 was considered as significance level. Results Plasma TNFα, NFκβ and fecal SP was reduced in all treatment groups compared to placebo group, however the reduction level was statistically significant in all three treatment groups for NFκβ and SP (P<0.001), in I+D and P+P groups for TNFα (P<0.003 and 0.002 respectively). There were no significant changes in TAC levels.

Conclusion administration of soy isoflavones alone or with vitamin D reduced plasma inflammatory markers and improved intestinal permeability index by lowering fecal SP activity.

P.A3.01.08

Alterations in natural Dendritic Cell (DC) subsets in advanced cancer patients

A. Kaur1, J. Adhikaree, H. Franks, P. Patel, A. M. Jackson

1Department of Cancer and Stem Cells, School of Medicine, University of Nottingham, Nottingham, United Kingdom.

The activity of natural DC is key for in vivo immunity and for the success of immune checkpoint blockade for advanced cancer patients. Nivolumab and ipilimumab block the inhibitory receptors namely PD-1 and CTLA-4 receptors on T cells allowing them to restore their anti-cancer functions. An understanding of the condition of natural DC subsets in these early stratification of responders, and may yield approaches to enhance the function of DC in other patients. We therefore measured the abundance and phenotype of 4 circulating DC subsets in cancer patients using a multi-parametric flow cytometry assay and compared it with data obtained from healthy controls. This assay has been performed for 16 patients with glioblastoma, 5 patients with advanced melanoma (currently on checkpoint inhibitor therapy) and 19 matched healthy donors. The average number of DC subsets in patient blood compared to healthy donor samples were 4476 vs 5056 of CD1c+ DC/mL, 193 vs 252 of CD141+ DC/mL, 1265 vs 2957 of plasmacytoid DC/mL (p-value=0.002) and 5760 vs 4265 of SLAN DC/mL respectively. A significant reduction in particular was observed in blood plasmacytoid DC subset in cancer patients as compared to healthy donors (P<0.05). As number of DC are important to efficiently activate the T cells after checkpoint inhibitor treatment, we believe that the number and phenotype of DC subsets may differ among responding and non-responding patients and this could further help identify the patients who would benefit the most with the therapy.

P.A3.01.09

Prognostic value of elevated T helper 17 and T helper regulatory cells related inflammatory serum markers in pathogenesis of septic polytrauma patients

S. Khurana1,2, M. Pusapati, J. Sarin1, J. Saxi1, L. Cortes1, E. Kolais1, R. P. Sekoly1, E. P. Ramphitith1

1CAPRON BIOSCIENCES INC., MONTREAL, Canada; 2University of Sao Paulo, Sao Paulo, Brazil; 3Case Western Reserve University, Cleveland, United States.

Dengue fever is the most prevalent mosquito-borne disease of humans, and is caused by the dengue virus (DENV) which is comprised of four serotype groups that circulate globally. Protective antibodies against one DENV serotype offer limited protection against the others. Therefore, a vaccine that protects against all four serotypes has been highly desirable. We analyzed plasma from a Phase II trial of TV003, a live-attenuated vaccine composed of all four DENV serotypes, to identify predictive biomarkers of vaccine efficacy. Pre-dose, days 6 and 15 post vaccination time points were analyzed from seronegative (n=17) or seropositive subjects (n=18). The samples were depleted of abundent proteins and processed to tryptic peptides for multiple reaction monitoring mass spectrometry (MRM-MS) using a previously defined assay composed of 298 host plasma proteins associated with the immune response. Overall protein detection rate was 96% with a study wide 9% median CV of the processes controls used and 71% of the targeted proteins were detected in >50% of the samples. Supervised and non-supervised study sample grouping and network analysis of the differentially expressed proteins was done. Oligovalent responders had consistently elevated expression of pro-inflammatory proteins at baseline and later time points compared to multivalent responders, and network analyses indicated that the IL6 and acute phase response pathways were induced. In contrast, multivalent responders at baseline had comparatively elevated cellular activation, proliferation, and migration as well as neutrophil function. These results suggest that baseline inflammation can reduce multivalent DENV serotype efficacy. Funded by NIH/NIAMD and the Butantan Institute.

P.A3.01.05

Protein biomarkers predictive of multivalent response to a novel dengue vaccine

V. Hindle1, P. Croteau1, S. Anir1, S. Lo1, J. Saxi1, L. Cortes1, E. Kolais1, R. P. Sekoly1, E. P. Parmitith1

1CAPRON BIOSCIENCES INC., MONTREAL, Canada; 2University of Sao Paulo, Sao Paulo, Brazil; 3Case Western Reserve University, Cleveland, United States.

POSTER PRESENTATIONS

142

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
A novel whole blood assay for detecting and profiling vaccine-induced Bordetella pertussis specific T-cells

N. Lambert, V. Carbone, J. van Goor, M. Djijit, E. Simonetti, K. van Schuppen, D. Diakovopoulos, A. Misjak, K. Mills, P. Verstreken, G. Berbers, C. van Els, F. Mascart

1National Institute of Public Health and the Environment, Bilthoven, Netherlands, 2Université Libre de Bruxelles, Brussels, Belgium, 3Radboud University Medical Center, Nijmegen, Netherlands, 4Trinity College Dublin, Dublin, Ireland.

Introduction: Pertussis, a severe respiratory infectious disease caused by the bacterium Bordetella pertussis (Bp), remains endemic despite vaccination. No correlates of protection have been identified yet. Bp-specific CD4+CD8+IFNγ+ T-cells are thought to play an important role in durable protection against infection and transmission. Novel sensitive and robust assays are needed to study the magnitude and differentiation of (vaccine-induced) antigen-specific T-cells in order to advance the development of improved pertussis vaccines.

Materials and methods: In a Dutch clinical study, 11-15 year-old healthy volunteers were boosted with an acellular pertussis vaccine. Whole blood samples, taken at day 0 (pre-booster), 14 and 28 (post-booster) were stimulated with Bp antigens overnight and production of cytokines was measured intracellularly and in supernatants by flow cytometry.

Results: Following vaccination, Bp-specific Th1 and/or Th2 cytokine producing T-cells increased to 0.02-0.5% of the CD4+CD3+ T-cell population. Supematant analyses are ongoing. Standardized analysis of flow cytometric data is being currently. Currently, inclusions are being finalized and overall results will be presented.

Conclusions: We have shown that our novel whole blood assay is capable of detecting low frequency T-cell responses against Bp. Further analysis is ongoing to assess the Th17/Th2 Th17 profiling capacity of the assay and whether different T-cell profiles can be detected in booster responses of differently primed teenagers.

The PERISCOPE project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115910. This Joint Undertaking receives support from the European Union’s Horizon 2020 research and innovation programme and EFPIA and BMS.

IFNy-preconditioning treatment increases immune system-related proteins in extracellular vesicles derived from human endometrial mesenchymal stromal cells

F. Marinaro, S. Sánchez-Margallo, V. Álvarez, R. Blázquez, E. López, B. Macías-García, M. Gómez-Serrano, J. C. Borjá, V. J. Vázquez, J. García-Casado

1Centro de Cirugía de Mínima Invasión, Cáceres, Spain, 2Centro Nacional Investigaciones Cardiovasculares CNIC, Madrid, Spain.

Introduction: Endometrial Mesenchymal Stromal Cells (endMSCs) are multipotent cells with clear immunomodulatory effects. These cells release extracellular vesicles (EV- endMSCs), although their role in immune and inflammatory response is still under investigation. Here we show the proteomic profile of EV-endMSCs together with the effect of IFNy-preconditioning.

Materials and Methods: EndMSCs were isolated from human menstrual blood, expanded in vitro and characterized by flow cytometry (n=3). EndMSCs were treated with IFNy (10ng/ml) for 6 days. IFNy was removed and supernatants were collected after 4 further days. EV-endMSCs were isolated from supernatants and protein extracts underwent high-throughput multiplexed quantitative proteomics based on iTRAQ labeling and mass spectrometry analyses. Enrichment analyses, using Gene Ontology (GO) and Reactome databases, were performed to evaluate the functional classification of the proteins identified.

Results: EndMSCs expressed CD56, CD44, CD29, CD90, CD105 and CD73 stemness markers, while EV-endMSCs expressed the exosome-related proteins CD9 and CD63. In the proteomic profiling, 1802 proteins were identified and 856 proteins belonged to GO term “extracellular vesicle” (p<0.01). Quantitative analyses showed 55 upregulated proteins after treatment (p<0.05). Enrichment analyses revealed that 26 proteins were associated to the innate immune response (i.e. M-CSF, IL12, STAT1) and 11 proteins to the Adaptive Immune Response (i.e. Cathepsin S, CD166 antigen, Beta-2-microglobulin). Moreover, 23 out of the 55 proteins were also related to the Reactome category “Immune system”.

Conclusions: EV-endMSCs proteomic profile reveals that IFNy treatment upregulates immune system-related proteins, revealing their therapeutic potential for the treatment of immune-mediated diseases.

Fecal calprotectin interlaboratory comparisons

M. Martin, A. Teniente-Serra

1Institute for Inflammation Research, Center for Rheumatology and Spine Disease, section 7521, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark, 2Hospital Universitari Vall d’Hebron, Barcelona, Barcelona, Spain.

Calprotectin is a dimer of calcium binding proteins mainly present in neutrophil’s cytosol. Elevated fecal calprotectin happens as a consequence of the migration of neutrophils to the intestinal mucosa due to any intestinal inflammation. Taken together with clinical signs and symptoms, this non-invasive test helps avoiding colonoscopies. Two rounds of an interlaboratory comparison were run along 2017, shipping stool aliquots from 4 different patients to 21 clinical labs. Samples were given after informed consent in HU Marques de Valdecilla. Each participant lab analyzed samples by their routine method. Both qualitative and quantitative results were reported. Robust statistics for interlaboratory comparison were performed following guidelines in ISO 13528. Three out of the 4 samples were assigned positive by consensus of at least 75% of participants, whereas the 4th sample remained inconclusive so far only 47% of labs reported it. It was reported to contain 280 μg/g calprotectin. Samples yielding positive results were reported to 344-3819 μg/g calprotectin. Some patients have been reported to have slightly raised levels of fecal calprotectin, who could only need monitoring. Those of them suffering from a low-grade inflammatory bowel disease (IBD) will usually evolve to higher calprotectin levels. It will be therefore useful for labs and interlaboratory comparisons can help narrowing their grey zones.

Flow cytometry: identifying biomarkers for the diagnosis of organ-specific autoimmune diseases and response to treatment

A. Teniente-Serra, M. Fernández, B. Soldevilla, E. Pirzarro, C. Ramo-Tella, R. Pujol-Barrell, E. Martinez-Cáceres

1Institute for Inflammation Research, Center for Rheumatology and Spine Disease, section 7521, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark.

In the last years, the significant development in the field of biomedical research has lead to the need to define new biomarkers for diagnosis, prognosis or monitoring of diseases. Moreover, the field of flow cytometry has been transformed from a tool that only looked at high-throughput flow cytometry panels and exclusive lymphocyte subpopulations to a tool for the description of small populations of cells. For this purpose we designed an exhaustive flow cytometry panel which allows to analyse minor lymphocyte subpopulations in peripheral blood, and validated it in several autoimmune diseases:

1) In Graves’ disease patients, a different pattern of lymphocyte subpopulations was identified compared to those without autoantibodies. For this purpose we designed an exhaustive flow cytometry panel which allows to analyse minor lymphocyte subpopulations in peripheral blood, and validated it in several autoimmune diseases:

1. We found changes in peripheral blood lymphocyte compartments of type 1 diabetes patients at onset of the disease.
2. In Graves’ disease patients, a different pattern of lymphocyte subpopulations was identified in patients clinically stable who maintain the presence of anti-TSH autoantibodies.
3. In patients with rheumatoid arthritis, we found a different pattern of lymphocyte subpopulations compared to those without autoantibodies.
4. In patients with chronic heart failure, we found a different pattern of lymphocyte subpopulations compared to healthy controls.

3) In multiple sclerosis, we found changes in lymphocyte subpopulations identified in untreated relapsing-remitting patients and progressive forms compared with healthy donors. The influence of immunomodulatory therapies on lymphocyte subpopulations was also assessed in a cross-sectional study.

Analyzing the influence of therapies on lymphocyte subpopulations we identified in a prospective study that Multiple sclerosis patients treated with fingolimod had different patterns of subpopulations, able to discriminate responders versus non-responders to the therapy, in a 12 month follow-up.

In conclusion, characterization of minor lymphocyte subpopulations in peripheral blood, by multiparametric flow cytometry, is a useful tool to identify potential biomarkers for the diagnosis and response to treatment of organ-specific autoimmune diseases, and by extension to other immune-mediated diseases.

Peptidylarginine deiminase 4 gene polymorphisms associate with systemic lupus erythematosus and lupus nephritis


1Institute for Inflammation Research, Center for Rheumatology and Spine Disease, section 7521, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark.

2Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

3Section for Periodontology, Department of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

4Copenhagen Lupus and Vasculitis Clinic, Center for Rheumatology and Spine Diseases, section 4242, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark.

Objective: Depression of immune complexes containing DNA and nuclear proteins, released during neutrophil extracellular trap (NET) formation, plays a key role in the pathogenesis of systemic lupus erythematosus (SLE) and lupus nephritis (LN). Histone citrullination, catalyzed by peptidylarginine deiminase 4 (PDA4), allows chromat development, and is thereby essential for NETosis. We sought to determine if selected single nucleotide polymorphisms (SNPs) in the encoding gene, PDA4, previously known to influence expression and functionality of the enzyme, affect risk of developing SLE and LN. Methods: 234 patients and 484 controls were genotyped for nine PDA4-SNPs using an in-house assay. Association of each SNP with organ involvement and gender: Results: Homozygosity and heterozygosity for rs1635564(T) were associated with increased SLE occurrence (P=0.02, OR 1.52, 95% CI 1.06-2.19 and P=0.03, OR 2.06, 95% CI 1.08-3.93, respectively), and homozygosity for rs1635564(T) was associated with increased LN occurrence (P=0.03, OR 3.35, 95% CI 1.2-10.97).

Notably, a gene dose effect for rs1635564(T) was observed for SLE and LN (P<0.005, OR 1.47, 95% CI 1.12-1.93 and P=0.01, OR 1.74, 95% CI 1.13-2.68, respectively). Additionally, minor allele carriage of five other SNPs (rs11203366, rs11203367, rs748811, rs2240340 and rs11203368) in high linkage disequilibrium, was associated with increased occurrence of LN and hypertension. Conclusion: We provide the first indications that the rs1635564 polymorphism of PDA4 could be a risk factor for SLE, in general, and LN in particular. Additional PDA4 polymorphisms may confer increased risk of LN. Overall, these findings support the notion of a role for citrullination in SLE and LN pathogenesis.
**Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands**

**P.A3.01.15**

Significant CD4+CD39+ T lymphocyte frequencies are differentially altered in patients with leukemia and autoimmune thrombocytopenia

N. Konova1, S. Metevel1, N. Kikadze1, G. Giorgobian1, T. Chikovani1, N. Jankishvili1, N. Jankishvili1
1Department of Immunology, Tbilisi State Medical University, Tbilisi, Georgia; 2Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia; 3Department of Surgery, Tbilisi State Medical University, Tbilisi, Georgia.

Introduction: Accumulating evidence suggests that the expression of ectonucleotidase CD39 in CD4+ T lymphocytes indicates on their suppressive activities. In certain immune pathologies - blood cancer or autoimmune hematological disorders, there is a direct relationship between circulating and spleenic T cell biomarkers reflecting the response to therapeutic interventions. The study aims to explore the differential expression of CD39 and FoxP3 in blood and spleenic CD4+ T cells of patients with chronic myelogenous leukaemia (CML) and immune thrombocytopenia (ITP). Individuals undergoing splenectomy due to other reason than cancer or autoimmune disease are used as controls.

Methods: Mononuclear cells from peripheral blood and dissociated spleen tissue were purified and the expressions of CD39 and FoxP3 were quantified within the CD4+ compartment of T lymphocytes. Data are acquired on a FACS Calibur flow cytometer and analyzed using FlowJo v10 software.

Results: Our data suggest that the frequency of total circulating as well as spleenic CD4+ T lymphocytes are comparable between CML and ITP patients and controls. However, CD39 expression is elevated in these T cells in patients with myelogenous leukemia and diminished in patients with ITP. Importantly, the differential expression of CD39 resembles FoxP3 levels in both groups of patients and is more evident in spleen compared to blood.

Conclusion: Expression of CD39 in spleenic CD4+ T cells can be considered as a good biomarker and, respectively, a prospective therapeutic target in patients with CML and ITP undergoing splenectomy.

**P.A3.01.16**

Granzyme B induced by RV0140 antigen better classified latently infected from active tuberculosis patients

R. Oumi1, A. Braiek1, V. Dirix1, H. Gharsali2, A. Jarrous3, N. Sendi1, A. Baccouche1, A. Akremi1, L. Gharbi-Douik1, R. Barbuco1, C. Benabdessalem2
1Laboratory of Transmission control and immunobiology of infections, Institut Pasteur de Tunis, Tunisia; 2Faculty of sciences of Bizerte, University of Carthage, Bizerte, Tunisia; 3University of Tunis El Manar, Tunis, Tunisia.

Introduc: The current disease is to identify markers that better classified LTBI from aTB. It has been shown that RV0140, a reactivation-associated antigen of Mtb, induced significantly higher IFNg in LTBI individuals as compared to aTB. Herein, we described that RV0140 induces high GranzymeM level mainly by PBMC derived from LTBI (n=33) as compared to aTB (n=18). ROC curves were used to evaluate the capacity of RV0140 specifically induced IFNg and Granzyme levels to classify LTBI from aTB. The results showed that, in response to RV0140, Granzyme allowed better discrimination of LTBI from aTB with areas under the curve (AUC) of 0.88 (95% CI 0.77-0.98) as compared to IFNg, AUC of 0.85 (95% CI 0.74-0.96). When combining GranzymeM and IFNg the AUC reaches 0.96. We show that GranzymeM could be considered as another biomarker of LTBI that could be used as an alternative or in conjunction of IFNg to better classified LTBI from aTB.

**P.A3.01.17**

Long-term immunologic effects of human endotoxemia: similarities and differences with sepsis

Y. Rodriguez Rosales,1 M. Koo,2 E. van Rijssen,3 M. van Weele,3 P. Pikkers,1 J. Leosten,3 H. Koenen3
1Laboratory of medical immunology, Radboudumc, Nijmegen, Netherlands; 2Intensive care unit, Radboudumc, Nijmegen, Netherlands.

Introduction: Septis is the cause of more than 5.3 million deaths per year, and novel immunotherapeutic strategies are highly warranted. Human models that mirror sepsis immunological effects and tissue response in humans during the first 24 hours, captures many hallmarks of the inflammatory response observed in early sepsis. However, the long-term immunologic effects of human experimental endotoxemia have been sparsely studied and could be determinant for the use of this human model in sepsis research therapy. Aim and methods: We studied the immune cell composition of healthy subjects challenged with a bolus of endotoxin [1 ng/kg] 4 hours, 2 days, and 10 days post-administration by flow cytometry to study the effects on the innate and adaptive immune system, and compared it with the immune cell composition in patients during the first 9 days after onset of septic shock. Results: As a result of experimental endotoxemia, an increase in absolute numbers of intermediate monocyte was observed, which also showed lower HLA-DR expression 20 days post-endotoxin. These changes differed with those observed in septic shock patients. Another long-term effect of experimental endotoxemia was evaluated numbers of effector CD8+ cells and an increased percentage of proliferating and cytokine expressing CD8+ cells, these phenomena were also present in sepsis patients. Conclusion: We propose that experimental endotoxemia can be used to study several aspects of adaptive immunity in sepsis, specifically the behavior of CD8+ T-cells, which may eventually aid the development of new therapies for sepsis patients.

**P.A3.01.18**

Clinical, demographic and laboratory data associated with the risk of progressive multifocal leukoencephalopathy risk in multiple sclerosis patients treated with natalizumab


Introduction: Natalizumab is an effectiverelapse-remitting multiple sclerosis (MS) treatment. However, the risk of progressive multifocal leukoencephalopathy (PML) is associated with high IC virus load in the absence of antibodies level risk. We aimed to identify new biomarkers predicting PML onset in patients treated with natalizumab. Patients: We studied 1240 MS patients treated with natalizumab. Thirty-five developed PML. Clinical and demographic variables were studied. Moreover, lipop-specific oligoclonal IgM bands (LSC-OMCB) were explored in a sub-group of 277 patients by isoelectric focusing and western blot. Data were analyzed with the Stata statistical package. We used Mann Whitney U tests, ROC curves, and uni and multi variate logistic regression analyses to study PML risk. Results: We confirmed that anti-IC antibodies predicted PML risk. However, no effect of prior immunosuppression or time on natalizumab were observed in this cohort. New variables were identified in our study. The strongest association with PML risk were a relapse rate below 0.5 prior to natalizumab (p<0.0001) and an age over 45 years (p=0.048). Those were the only variables that remained significant in the multivariate analysis of the total cohort (AUC=0.85). We also ascertained the association of LSC-OMCB and lower PML risk (p=0.0001). In conclusion, relapse rate below 0.5 and age over 45 constituted an accurate model for PML risk stratification (AUC=0.92). Conclusion: We propose here a simple score that can statistically contribute to establish PML risk in individual MS patients treated with natalizumab.

**P.A3.01.19**

Mass cytometry and immune cell specific gene expression analysis of matched psoriatic arthritis blood and synovial fluid

N. Yager,1 S. Cole,1 A. Lledo Larra2, A. Maroof,3 C. Simpson,4 P. Bowes,1 H. Al-Mossawi2
1University of Oxford, Oxford, United Kingdom; 2UCB Pharma, Slough, United Kingdom.

Introduction: Mass Cytometry (CyTOF) has revolutionised the way cell samples can be immunophenotyped, allowing simultaneous quantification of >30 parameters with minimal spillover. This study aims to use CyTOF to generate a high-dimensional data-set of immune populations in matched psoriatic arthritis (PsA) blood and synovial fluid directly ex vivo, followed by transcriptomic analysis of key expanded cell populations. Materials and Methods: Paired blood and synovial fluid were taken from 10 PsA patients and fixed with formaldehyde within 30 min of venipuncture/aspiration. The cells were stained with a 34-channel CyTOF panel of surface markers. Analysis was performed using conventional biaxial manual gating and unbiased visualisation methods including PHAEGraph and SPADA. RNA was isolated from the core expanded populations of freshly cell sorted PBMC and synovial fluid samples (n = 3), and a 348-gene array was performed. Results: Using only one staining panel and applying multiple clustering algorithms, we were able to observe distinct changes in cell populations structure in the synovial fluid of PsA patients compared to the blood. While some populations diminished (e.g. B cells), others expanded, including memory T cells and CD14 myeloid populations. Gene expression analysis of these expanded populations demonstrated multiple significant differences between the blood and synovial fluid that was shared across PsA patients. Conclusions: We have generated an in-depth map of the immune landscape in PsA blood and matched synovial fluid in combination with a cell-specific transcriptomic analysis of expanded synovial cell populations to reveal novel inflammatory modules in PsA pathogenesis.
Compared to healthy controls, lysing protocol. cells were evaluated after staining with anti-CD5, -CD19, -CD3, -CD4, -CXCR5, and intracytoplasmic BATF monoclonal antibodies using flow cytometry according to whole blood and a reference gene, of BATF is not known in CLL pathogenesis. In this study, BATF mRNA and protein expressions in CLL were investigated. Peripheral blood samples were collected from 37 patients with CLL and 16 healthy subjects. Total RNA was isolated from whole blood samples and cDNA synthesis was done. BATF and a reference gene, HPRT1, were analyzed by real-time PCR. The relative expression levels were calculated by using ΔC, method. CLL-B (CD5’CD19’ and Tfol (CD3’CD4’CXCR5’)) cells were evaluated after staining with anti-CD5, -CD19, -CD3, -CD4, -CXCR5, and intracytoplasmic BATF monoclonal antibodies using flow cytometry according to whole blood lysing protocol. Compared to healthy controls, BATF mRNA levels (p<0.05); BATF and BATF’CD19’ cells (p=0.0013 and p<0.0001, respectively); BATF expression in both CD19’ and CD3’ cells (p=0.0011 and p=0.003, respectively); CD3’CD4’CXCR5’ cells (p=0.007) were increased in patients with CLL and also high BATF expression of CD3’CD4’CXCR5’ Tfol cells (p=0.001) were found. Increased BATF expression of different lymphocyte subsets in patients with CLL was observed. Given the current role of BATF with some molecules associated with the pathogenesis of CLL, our findings provide the impression that BATF could play a role in the biology of CLL.

P.A3.02.01

Complement factor H increased and associated with elevated oxidative stress markers and IL-1β in Algerian Behcet’s disease patients. A. Chekroun1, H. Belguedouz2, K. Lahmari1, M. Terouh1, F. Z. Mazari1, F. Otmani1, D. Hakemi1, C. Fouli-Boujaouf1
1Institute of Medicine, University of Tlemcen, Algeria; 2UCL bioethics department, CHU Nfiss Hammoudi, Algiers, Algeria; 1Institute of medicine department, CHU Bab ElOued, Algiers, Algeria.

Background: Behcet’s disease (BD) is a multisystem disease. It stands at the crossroad between autoimmunity and autoinflammatory disorders. In the present study, we aimed to assess the plasma level of complement factor H (CFH) and elucidate its possible correlation with oxidative stress markers and the proinflammatory cytokine IL-1β in Algerian BD patients.

Patients and methods: We investigated the CFH, stress oxidative markers (Nitric oxide (NO), Advanced oxidized proteins products (AOPP)) and IL-1β in Algerian BD patients (78: Active BD patients (ABP, 28) and Inactive BD patients (IBP, 50) referring to disease activity and clinical manifestations compared to healthy controls (HC, 41). Mann–Whitney U and Pearson correlation tests were used for statistical analyses.

Results: CFH levels significantly increased in ABP and IBP (p<0.0001) compared to HC, whereas there is no significant difference (p=0.05) between ABP and IBP and NO and AOPP levels significantly increased in ABP (p<0.001) compared to IBP and HC and in IBP (p=0.001) versus HC. ABP displayed higher plasma levels of IL-1β (p=0.05) versus IBP and HC (P<0.0001), also IBP showed higher levels of IL-1β (p<0.01) versus HC.

Conclusion: Our study highlights an overexpression of CFH correlated with high levels of oxidative stress markers and IL-1β. We suggest to further study this relationship to illuminate alternative paths of therapeutic in BD.

P.A3.02.02

Analysis of the immune system in patients with hereditary hemochromatosis V. Bönnemann, M. Claus, P. Bröde, K. Golla, C. Watzi; IfDaG, Dortmund, Germany.

Hereditary Hemochromatosis (HH) is an autosomal recessive disorder of the iron metabolism. The typical systemic iron overload in this disease can cause dysfunction of several organs by iron accumulation. Iron is crucial for cell function, but on the other hand, it produces reactive oxygen species (ROS) by the catalysis of important chemical reactions. Since ROS are known to cause oxidative stress and cellular damage, a precise regulation of iron within cells is necessary. The identification of the HFE gene was a major breakthrough for the understanding of the HH. This gene encodes for a novel major histocompatibility complex class 1-related molecule, which play important roles in the immune system. Due to the fact that some HH patients showed aberrant NK cell functionalities in preliminary studies, we wanted to examine whether NK cells could be influenced by iron overload in HH patients. To investigate the properties of HH NK cells, PBMC of hemochromatosis patients and age-matched controls were used for immunophenotyping and functional assays such as degranulation and chromium-release assay. In addition, a cytometric bead array and a ferritin ELISA were performed. We observed increased basal and stimulated production of pro-inflammatory cytokines, assuming a distinct functionality of HH PBMC compared to controls. In addition we did not find aberrant NK cell phenotypes, but a general decrease of total granulocyte numbers. These data underline the complexity and sensitivity of the immune system to systemic influences.

P.A3.02.03

The expression profile of the ubiquitin-like modifier FAT10 in immune cells suggests cell type-specific functions R. Schreger1, M. M. Maih, S. Müller, F. Brackmann2, A. Aichern1, M. Basler1, M. Greutert1, 1Biotechnology Institute Thurgau, Kreuzlingen, Switzerland.

The TNF and IFN-y-inducible ubiquitin-like modifier HLA-F adjacent transcript 10 (FAT10) is most prominently expressed in immunological tissues but information regarding basal expression and inducibility of FAT10 in the different types of immune cells is still lacking. Hence, we investigated FAT10 mRNA expression in the major human and murine immune cell subsets, and FAT10 protein expression in human leukocytes. We isolated the different human leukocytes from peripheral blood and the murine immune cell subsets from spleen. The purified leukocytes were left untreated or stimulated with TNF and IFN-γ or LPS to induce FAT10 followed by quantitative real-time PCR or western blot analysis. Basal expression of FAT10 mRNA and protein was generally low but strongly up-regulated by IFN-γ and TNF in all immune cell subsets. LPS treatment induced FAT10 expression marginally in human CD8+ T cells and murine granulocytes, but it increased Fat10 expression significantly in murine regulatory T cells. Yet, in human CD8+ T cells, natural killer cells, natural killer T cells, and dendritic cells, the FAT10 mRNA was expressed without induction. Similarly, murine macrophages, monocytes, and regulatory T cells expressed Fat10 in the absence of stimulation. In summary, our findings suggest particular functions of FAT10 in these cell types. Furthermore, we observed not only a cell type-specific but also a species-specific basal FAT10 expression profile. Our data will serve as a guideline for future investigations to further elucidate FAT10’s role in the immune system.

P.A3.02.04

Personalized monitoring of immune system from undifferentiated arthritis to rheumatoid arthritis in humans and during treatment - a possible practical usage of cytokine profile E. Bryl1, E. Brzezieniec1, I. Bzoma1, M. Bykowski1, M. Szarecka1, A. Daco1, J. M. Witkowski2; 1Department of Pathology and Experimental Rheumatology, Medical University of Gdańsk, Gdańsk, Poland, 2Pomeranian Center of Rheumatology, Szapot, Poland.

Introduction. Undifferentiated arthritis (UA) being usually the first clinical representation of many forms of specific arthritis mostly progresses to rheumatoid arthritis (RA). The immunological studies in established RA showed the profound changes in peripheral blood CD4+ T cells and changed the paradigm of RA being a joint disease only. Our goal was to monitor the cytokine profile in patients progressing from early UA to RA and being monitored during treatment.

Patients and methods: The group of UA patients developing RA (UA→RA) was identified from a total of 121 people with arthralgia. All subjects underwent clinical and laboratory evaluation, including acute phase reactants (APR) and autoantibodies (anti-CCP, RF, ANA-HEp-2). Cytokines IFN-γ, IL-10, TNF, IL-17A, IL-6, IL-18, and IL-2 in sera and mononuclear cell supernatants were assayed using BD® CBA Flex Sets. UA→RA patients were followed up for six months since the final RA diagnosis and DMARDs treatment.

Results: 34.3% of patients with UA developed RA. We observed specific cytokine patterns characterizing each patient, which altered during course of disease. We distinguished three UA→RA cohorts with different cytokine profiles: the group of patients susceptible to the therapy, the group refractory to the therapy and the group with variable responses to the therapy.

Conclusions: The serum cytokine profiles change in the course of RA and may be potentially used for optimization of RA monitoring. The personal profile including multiplexed cytokine patterns in serum and supernatant may be likely utilized for optimization of therapy introduction and monitoring.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
POSTER PRESENTATIONS

Fetal calcium of Gal-3 was enhanced in CRC patients with higher nuclear grade, poor tumor tissue differentiation, advanced TNM stage and metastatic disease. Gal-3/TNF-α ratio was significantly higher in CRC patients with a higher trend in patients with adenocarcinoma. Positive correlation between fecal Gal-3 and disease severity, tumor progression and biomarkers ACP and CEA, respectively was also observed. Predominance of Gal-3 in patients with advanced disease may implicate in its role in limiting ongoing proinflammatory processes. The fecal values of Gal-3 can be used as valuable marker for CRC severity and progression.

P.A.3.02.12 Complement component C1q as serum biomarker to detect active tuberculosis

1-Leiden University Medical Center, Leiden, Netherlands, 2-Medical Research Council Unit, Banjul, Gambia, 3-National Institute for Infectious Diseases, Rome, Italy, 4-Biomedical Primate Research Centre, Rijswijk, Netherlands.

Introduction: Tuberculosis (TB) remains a major threat to global health. Currently, diagnosis of active TB is hampered by the lack of specific biomarkers that discriminate active TB from other (lung) diseases or latent TB infection (LTBI). The complement system is an important part of the innate immune system and integrated gene expression analyses have revealed that complement genes, in particular the C1q genes, were expressed at higher levels in active TB compared to LTBI.

Methods: C1q protein levels were determined using ELISA in sera from patients, from geographically distinct areas, with active TB, LTBI as well as disease controls.

Results: Serum levels of C1q were increased in active TB compared to LTBI in four independent cohorts and discriminated with an AUC of 0.77 [0.70; 0.83]. After six months of TB treatment, levels of C1q had normalized to those of endemic controls, indicating an association with disease rather than individual genetic predisposition. Importantly, C1q levels in sera of TB patients were significantly higher compared to patients with sarcoidosis or pneumonia, clinically differential diagnoses. Moreover, exposure to other mycobacteria such as M. leprae (leprosy patients) or BCG (vaccines) did not present with elevated levels of serum C1q. In agreement with the human data, in non-human primates challenged with M. tuberculosis, tubulin and C1q levels were upregulated in infected animals.

Conclusions: Circulating C1q is a novel TB biomarker, which discriminates active TB from most other conditions, including other lung diseases, and could have added value in diagnosing TB.

P.A.3.02.13 Cellular immune response to T3SS proteins in humans vaccinated with live bacterial vaccine

A. M. Lypina1, V. A. Fedorova2, S. S. Zaitsev3, M. A. Khizhnyakova2, L. V. Sayapina1, M. V. Tlepneva1, O. V. Ulianova1, E. P. Lyapina2, S. S. Ulyanov2, V. L. Motin1;
1-Laboratory for Molecular Biology and Nanobiotechnology, Federal Research Center for Virology and Microbiology (FRCVIM), Branch in Saratov, Saratov, Russian Federation, 2-Department for Molecular Biology, Biotechnology and Chemistry, Saratov State Agrarian University named after N.I. Vavilov, Saratov, Russian Federation, 3-Department of Vaccin Control, Scientific Center on Expertise of Medical Application Products, Moscow, Russian Federation, 4-Department of Pathology, Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, United States, 5-Department for Infectious Diseases, Saratov State Medical University named after V.I. Razumovsky, Saratov, Russian Federation.

Type III Secretion System (T3SS) is a special “nanomachine” widely used by Gram-negative bacteria for the delivery of effector proteins to mammalian target cells. In fact, T3SS components may participate in eliciting effective adaptive response. In this study we investigated human cellular immune response to the live plague vaccine (LPV), an attenuated Y. pestis EV strain line NIIEG possessing T3SS. Highly pure T3SS recombinant proteins, LcrV and YopM, were used for the in vitro stimulation of PBMCs collected from multiply immunized donors (n=18) and control naive individuals (n=6) to assess lymphocyte proliferation and Th1/Th2/Th17 polarization. We found that although there was no significant difference between vaccinated and control groups, proliferative response to all antigens, re-stimulation with YopM induced a high proliferative reaction of lymphocytes from vacccinees exceeding those with control F1 antigen (p<0.05) and LcrV (p<0.001) 2.1-fold and 3.3-fold respectively. Marked proliferative response to YopM was accompanied by high production of IFN-γ and TNF-α, which was specific for vaccinees (p<0.05). On the contrary, F1 stimulus specifically induced mixed Th1/Th2 response with IFN-γ, TNF-α and IL-4 secretion in vaccinees (p<0.05). In vitro stimulation with LcrV induced an increased secretion of IFN-γ exclusively in vaccinated persons (p<0.05), while the similar response to YopM and F1 was nonspecific (p>0.05). In conclusion, we have shown that T3SS effector protein YopM, but not LcrV, is likely to be strongly involved in the cellular response elicited by LPV in humans. This antigen-specific response was Th1-polarized. This study was supported by the RFFR #18-016-0159.

P.A.3.02.14 IL-10 and IL-17 chronic low grade inflammation marker in colorectal cancer patients and healthy elderly

I. Pantsulaia, A. Aladashvili, M. Jobadze, N. Kikadze, T. Atamashvili, T. Chikovani;
1-Vl.Bakhutashvili Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia, 2-Ilisi State Medical University, Tbilisi, Georgia.

Background: Permanent exposure of environmental factors and numerous interactions with pathogen leads to a chronic inflammatory state in older individuals. In fact, acute inflammation is a beneficial process but with ageing it becomes chronic and leads to tissues dysfunction or degeneration. Recently, many studies have been demonstrated that chronic inflammation is associated with all stages of cancer development increasing its risk, supporting and promoting cancer progression. Moreover preventive treatment with anti-inflammatory drugs like aspirin reduces the incidence and mortality for colorectal cancer. Consequently, evaluation inflammatory state in different tumors and age matched individuals is essential for designing new personalized treatments.

Methods: In this study 135 samples were collected from patients with colorectal cancer (n=103) and healthy elderly persons (n=32). Serum was obtained from colorectal cancer patients and healthy elderly individuals. IL-10 and IL-17 concentrations were significantly (P<0.01) decreased in elderly group comparing to young people. On the contrary to IL-10, IL-17 levels were higher in aged persons than young population. The correlations among studied cytokines were strong and significant in both groups. Conclusion: The outcomes of study suggest a shift the inflammatory status in elderly population, however, could not prove a clear and strong polarization. The ratio of IL-10/IL-17 concentrations should be used as indicator of declining health in aged individuals.

P.A.3.02.15 Utility of serum levels of interleukin 13 in patients with sarcoidosis.

P. Sohal1, K. Upadhyay2, A. Ali2, H. Agarwalla2, M. Goel2, V. Singh1, K. Madan2, A. Mohan3, R. Guleria2;
1-Pulmonary Medicine and Sleep Disorders, AIIMS, New Delhi, India, 2-Biochemistry, AIIMS, New Delhi, India, 3-Biostatistics, AIIMS, New Delhi, India.

Introduction: Cytokines are inflammatory mediators which play an important part in immunopathogenesis of sarcoidosis. Cytokine IL-13 produced by type 2 helper T cells (TH2), mast cells, eosinophils and basophils, is the key mediator during effector phase of allergic inflammation. Our study aimed to describe the variations of serum levels of IL-13 among sarcoidosis and healthy elderly controls.

Methods: In this study 57 newly diagnosed sarcoidosis patients and 32 healthy controls were enrolled. A total of 27 sarcoidosis patients were followed up for 6 months after initiation of treatment. Serum levels of IL-13 were measured using Enzyme-linked Immunossorbtant Assay (ELISA) among sarcoidosis patients at baseline, post six months of treatment and healthy controls. Results: Median (range) of serum IL-13 levels in patients with sarcoidosis at baseline, after treatment and in healthy controls were 16.12 (7.289-93.90)pg/ml, 14.81 (7.77-29.51)pg/ml and 11.59 (4.265-31.24) pg/ml respectively. The IL-13 levels were higher among patients at baseline compared to healthy controls (p<0.01). IL-13 levels were significantly reduced among patients after six months of treatment as compared to baseline (p<0.03). There was no significant difference in IL-13 levels after follow up compared to healthy controls. Conclusion: The data indicates that IL-13 is inflammation of sarcoidosis and can be a useful marker of disease activity. Serum IL-13 levels at baseline may significantly aid in diagnosis of sarcoidosis. To confirm this, large population study is warranted.

P.A.3.02.16 Antiinflammatory fluid complement component C3 and complement factor I levels in pregnancies complicated by the preterm prelabor rupture of membranes

A. Sourcek1, I. Musilova1, C. Andryl2, J. Krejsek3, M. Kacerovsky4;
1-Department of Clinical Immunology and Allergy, University Hospital, Hradec Kralove, Czech Republic, 2-Department of Obstetrics and Gynecology, University Hospital, Hradec Kralove, Czech Republic, 3-Biomedical Research Center, University Hospital, Hradec Kralove, Czech Republic.

Introduction: Preterm prelabor rupture of membranes (PPROM) is characterized by the rupture of fetal membranes and leakage of amniotic fluid before onset of regular labor activity prior to gestational age 37 weeks. It is a significant public health problem because it complicates about 3% of all pregnancies (about one-half of spontaneous preterm deliveries) and has a significant health, social and economic impact on society. Etiology of PPROM is not yet fully elucidated, but it is known that is commonly accompanied by microbial invasion of the amniotic cavity (MIAC) and/or intra-amniotic inflammation (IAI). Objectives: The aim of this study was to determine the antiinflammatory fluid complement component C3 and complement factor I concentrations in women with PPROM based on MIAC, IAI and microbial-associated IAI. Methods: One hundred fifty-nine women with singleton pregnancies complicated by PPROM were enrolled in the study. Single cord specimens were obtained by PPROM neonatal amniocentesis and were assayed for complement component C3 and complement factor I concentrations by ELISA tests. Conclusion: The presence of IAI and microbial-associated IAI is connected with higher concentrations of complement component C3 and lower concentrations of complement factor I. MIAC alone had no impact on C3 or complement factor I levels in amniotic fluid. The results show that complement may be involved in etiology of PPROM. Acknowledgments: This work was supported by the Faculty Hospital in Hradec Kralove and by Charles University in Prague, Faculty of Medicine in Hradec Kralove, Czech Republic, project "PROGRES Q40/10".

147

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.A3.02.17 Serum levels of BAFF and IL17 induce tissue damage in pauci immune small vessel vasculitis and glomerulonephritis

M. Stangou1, A. Fylaktou1, D. Daikidou1, C. Nikolaidou1, C. Stamou1, E. Sampani2, E. Moraiti2, A. Papagianni2;
1Department of Pathology, Aristotle University of Thessaloniki, Thessaloniki, Greece, 2Department of Pathology, General University Hospital of Alexandroupolis, Alexandroupolis, Greece.

Introduction: Renal impairment in small vessel vasculitis (SVV) is characterised by inflammation, proliferation and necrosis. B cell activating or B lymphocyte stimulating factor (BAFF/BlyS) and Interleukin 17 (IL-17) may be involved in the pathogenesis of tissue damage. Patients-Methods: BAFF and IL17 levels were measured by EUSA, in serum from patients with SVV and glomerulonephritis [13 females, age 57.5yrs (25-80)] collected at the day of renal biopsy, before any treatment was applied, and results were correlated with renal pathology and urinary excretion of IFN-γ, IL-10 and G-CSF. Treatment protocol consisted of prednisolone-cyclophosphamide as induction, with or without plasmapheresis, followed by prednisolone-saszumycin for at least 48 months. Results: Serum levels of BAFF and IL-17 were significantly increased in SVV patients compared to controls (1601.45±34.96 vs. 960.33±30.24ng/ml, \( p<0.001 \) and 21.71 ±25.9 vs. 12.43±8.3mg/l, \( p<0.009 \)) respectively. Both serum BAFF and IL-17 levels were significantly increased in ANCA+ patients (1663.5±1309ng/ml vs. 1073.85±251.8mg/ml, \( p=0.02 \)) and 24.9±30mg/l vs. 15.2±2.8mg/l, \( p=0.05 \) respectively. Serum BAFF levels correlated significantly with urinary IL-10 (\( r=0.04 \)) and INF-γ (\( r=0.03 \)), percentage of cellular crescents (\( p=0.04 \)), while serum IL-17 was correlated with urinary IL-17 levels (\( r=0.04 \)), degree of interstitial fibrosis and proteinuria (\( p=0.02 \)). Conclusions: BAFF and IL-17 are increased in the serum of patients with glomerulonephritis secondary to SVV, especially in the presence of ANCA+. BAFF at early stages may be implicated in inflammation, through the production of IL-10 and INF-γ, while IL-17 in more advanced stages, induces fibrosis and deterioration of renal function.

P.A3.02.18 Epidermal Growth Factor (EGF) as biomarker of renal function outcome in different forms of primary glomerular diseases.

M. Stangou1, A. Fylaktou1, E. Sampani2, C. Nikolaidou1, D. Daikidou2, E. Moraiti2, A. Papagianni2;
1Department of Pathology, Aristotle University of Thessaloniki, Thessaloniki, Greece, 2Department of Pathology, Hippokration Hospital, Thessaloniki, Greece, 3Department of Pathology, General University Hospital of Alexandroupolis, Alexandroupolis, Greece.

Introduction: and aims: EGF acts through EGF receptor and facilitates regeneration of tubular epithelial cells after injury. We investigated the role of urinary EGF excretion as biomarker for pathological and renal function outcome in glomerulonephritis.

Methods: EGF urinary levels were estimated in 3 forms of glomerular diseases: (1) IgA nephropathy (IgAN), as chronic glomerulonephritis, \( [n=50, 21\text{ female}, \text{age 39.8yrs}(18-65)] \), (2) pauci rapidly progressive glomerulonephritis (PRGN), as acute glomerulonephritis, \( [n=38, 17\text{ female}, \text{age 59.5yrs}(25-80)] \) and, (3) Nephrotic syndrome (NS) due to focal segmental glomerulosclerosis (FSGS) \( [n=23, 9\text{ female}, \text{age 47.5yrs}(19-79)] \) and minimal change disease (MCD) \( [n=12, 7\text{ female}, \text{age 45.5yrs}(37-62)] \).

Declining renal function was associated with reduced urinary EGF levels (\( p<0.005 \)) in IgAN (0.04±0.04 vs. 0.2±0.2mg/mgUcr, \( p<0.01 \)) and FSGS (0.007±0.004 vs. 0.6±0.04mg/mgUcr, \( p<0.01 \)). Serum levels of EGF correlated significantly with severity of interstitial fibrosis, \( r=-0.6, p<0.04 \), in IgAN, with the percentage of fibrous crescents, \( r=-0.6, p<0.01 \) in PRGN, and finally, with the percentage of global sclerosed glomeruli, \( r=-0.5, p<0.04 \), degree of fibrosis, \( r=-0.6, p<0.04 \), degree of interstitial fibrosis, \( r=-0.6, p<0.04 \) and degree of chronic tubular atrophy, \( r=-0.5, p<0.04 \) in patients with NS.

Conclusions: EGF urinary levels in patients with IgAN, PRGN and FSGS might be used as biomarkers of renal function outcome. EGF is a promising candidate for clinical studies and future clinical trials.

P.A3.02.19 Circulating cytokines predict response to anti PD1 therapy in NSCLC


Background: Despite the recent successes of immunotherapy in the treatment of non small cell lung cancer (NSCLC), only 20-30% of patients have a long-term benefit from immunotherapy, while the remaining 70-80% result resistant. The identification of responder patients represents the open issue of immunotherapy. In the context of several biomarkers the study of cytokine profile represents a promising approach. Chemokines, such as CXCL10 and sICAM, can facilitate chemotactic recruitment of TILs thus favouring their intratumoral trafficking accumulation and increasing the immune response against tumor. In the present study, we explored the prognostic impact of circulating cytokines in 18 NSCLC patients undergoing anti PD-1 treatment.

Methods: Sera from 18 NSCLC patients in treatment with nivolumab, were analysed using the ProcartaPlex Human Inflammation Panel. Samples were measured by BioPlex Magpix Multiplex Reader and data analysis was performed using BioPlex Manager MP software.

Results: Seven out of 18 patients presented early progression, defined as progression of disease within 6 months from the beginning of nivolumab treatment. The median value of CXCL10 and sICAM in patients with early progression, defined as progression of disease within 6 months from the beginning of nivolumab treatment, was 1339 pg/ml and 255202 pg/ml respectively, while in no progressor patients were 2334 pg/ml for CXCL10 and 370000 pg/ml for sICAM. We found a significant inverse association between the circulating levels of the chemokine CXCL10 and sICAM and early progression (\( p<0.05 \)). Conclusions: CXCL10 and sICAM seem to be associated with resistance to immunotherapy and could predict resistance to anti PD1-1 treatment. These preliminary results suggest the possibility of design and select alternative strategies to overcome the resistance in progressor patients.
POSTER PRESENTATIONS

P.A3.02.22
In depth analysis of antibody reactivities directed against numerous citrullinated peptides derived from the human proteome
H. Thiesen1, F. Steinbeck2, E. Schade3, S. Drynda4, J. Kekow5
1Institute of Immunology, Rostock, Germany; 2University of Applied Sciences in Hamburg, Germany; 3University of Hamburg, Germany; 4Helios Fachklinik Vogelsang-Gammern, Gammern, Germany; 5Helios Fachklinik Vogelsang-Gammern, Gammern, Germany.

Introduction: Our analysis is driven by the interest to select citrullinated peptides whose sequences are derived from the human proteome. Panels of peptides were designed that show differential epitope-antibody-reactivities (EAR) as initially found in intravenous immunoglobulin preparations, see PMID: 27039866 and PMID: 24244326. Methods: High throughput microwells representing pairs of peptides were incubated with sera of patients suffering from CCP positive versus CCP negative rheumatoid arthritis (RA). The paired peptides carry either an arginine, whereas the other sequence but the arginine replaced by a citrulline The most informative citrullinated peptides were then comparatively evaluated by applying MSD multi-array analysis. Ten different peptides placed per well were either incubated with sera of blood donors or of substratified RA patients. Results: In total, 96 different citrullinated peptides were finally characterized, leading to distinct subsets of human derived citrullinated peptides. One peptide panel has the potential to replace the commerical CCP assay, the other panel stratifies CCP negative RA patients and the third one is suitable to determine putative preclinical RA within blood donors. Conclusion: Our comparative computational analysis of the most informative citrullinated peptides specifies proteins that play major roles in conducting and eliciting immune responses as well as in performing cellular functions of intercellular communication. Our data set represents putative candidate peptides whose antibody reactivity profiles might have great values in determining clinical features being causative in initiation and progression of CCP positive RA disease.

P.A3.03 Immunomonitoring and biomarkers - Part 3

P.A3.03.01
The IgG4-IgG RNA ratio differentiates active disease from remission in granulomatosis with polyangiitis: A new disease activity marker?
A. Al-Soudi1, D. Daikidou2, L. Hafkenscheid3, P.A3.03.04, O. BELHIBA4
1Department of Internal Medicine, VU University Medical Center, Amsterdam, Netherlands; 2Department of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada; 3Department of Immunology, Rostock, Germany; 4Laboratory of Clinical Immunology, Allergy and Inflammation LICIA, Faculty of Medicine and Pharmacy Hassan II University, Casablanca, Morocco, Casablanca, Morocco.

Introduction: Our analysis is driven by the interest to select citrullinated peptides whose sequences are derived from the human proteome. Panels of peptides were designed that show differential epitope-antibody-reactivities (EAR) as initially found in intravenous immunoglobulin preparations, see PMID: 27039866 and PMID: 24244326. Methods: High throughput microwells representing pairs of peptides were incubated with sera of patients suffering from CCP positive versus CCP negative rheumatoid arthritis (RA). The paired peptides carry either an arginine, whereas the other sequence but the arginine replaced by a citrulline The most informative citrullinated peptides were then comparatively evaluated by applying MSD multi-array analysis. Ten different peptides placed per well were either incubated with sera of blood donors or of substratified RA patients. Results: In total, 96 different citrullinated peptides were finally characterized, leading to distinct subsets of human derived citrullinated peptides. One peptide panel has the potential to replace the commerical CCP assay, the other panel stratifies CCP negative RA patients and the third one is suitable to determine putative preclinical RA within blood donors. Conclusion: Our comparative computational analysis of the most informative citrullinated peptides specifies proteins that play major roles in conducting and eliciting immune responses as well as in performing cellular functions of intercellular communication. Our data set represents putative candidate peptides whose antibody reactivity profiles might have great values in determining clinical features being causative in initiation and progression of CCP positive RA disease.

P.A3.03.02
Research of Anti-GAD and Anti-IA2 autoantibodies by ELISA test in a series of Moroccan pediatric patient with diabetes type 1
G. BELHRA1, A. Fylaktou2, D. Asouchidou3, T. Chronis4, D. Daikidou5, E. Schade6, B. Ahmed Aziz7
1University of Medicine and Pharmacy Hassan II University- Casablanca, Morocco, Casablanca, Morocco; 2Pediatric Endocrinology Unit, Hospital d’enfant Abderrahim Harouchi Chu Ibn Rochd, Casablanca, Morocco, Casablanca, Morocco; 3Laboratory of Clinical Immunology, Allergy and Inflammation LICIA, Faculty of Medicine and Pharmacy Hassan II University, Casablanca, Morocco, Casablanca, Morocco; 4Clinical Immunology Unit, Infectious Disease, Hospital d’enfant Abderrahim Harouchi, CHU Ibn Rochd, Morocco, Casablanca, Morocco.

Introduction: Type I diabetes (T1D) is an autoimmune disease with a symptomatic period characterized by the destruction of insulin-producing β cells. This preclinical phase is of a variable duration during which various autoantibodies are generated against several beta cell antigens such as : Anti Glutamate Acid Decarboxylase (Anti-GAD), Anti Tyrosine Phosphatase (Anti-IA2). Objectives: In this work, we want to evaluate the diagnostic value of Anti-GAD and Anti-IA2 antibodies in a series based on 78 Moroccan subjects initially under 16, suspected T1D. Methods and Results: Our study concerns 78 children aged from 1 to 16 years followed for an evocative table of T1D. Samples of patients were analyzed by ELISA tests using Anti-GAD and Anti-IA2 autoantibody kits (EUROIMMUN). Results and Discussion: Our series consists mainly of 74% of newly diagnosed patients for T1D and 26% of confirmed diabetic patients, of whom 52% are females. The mean age of diagnosis is 7 ± 4 years, the mean of HbA1c at the time of diagnosis is 11.63 ± 2.16%, and the percentage of family history in our series is 60%. The proportion of positive results for Anti-IA2 antibodies and Anti-GAD antibodies are respectively 76.92% and 62.82%, and 52.56% of patients are positive for both autoantibodies. Conclusion: This study confirmed the diagnosis and the classification of T1D (type 1A) in 87.18% of patients, and we reported that the prevalence of Anti-GAD and Anti-IA2 is higher in girls than in boys.

P.A3.03.03
Analysis of B cell lymphocyte subpopulations in pre and post dialysis end stage renal disease patients
D. Daikidou1, A. Fylktou2, M. Stangou3, D. Asouchidou3, T. Chronis4, B. Ahmed Aziz5
1Department of Nephrology, Aristotle University of Thessaloniki, Thessaloniki, Greece; 2Department of Immunology, National Peripheral Histocompatibility Center, Hippokration Hospital, Thessaloniki, Greece.

Introduction and aims: End-stage renal disease (ESRD) shows an immunodeficiency, which makes a significant contribution to morbidity and mortality. The present study aims at analysis of B lymphocyte subpopulations in pre- and six months post-dialysis ESRD patients. Methods: B cells (CD45+ CD19+) and their subsets B1a (CD19+CD5+), naive (CD19+ CD27–), memory (CD19+ CD27+), CD19+ Baff+ and CD19+ Igm+, were quantified using flow cytometry in the peripheral blood of 27 pre-dialysis and 11 post-dialysis patients. The results were compared to healthy control group. Results: ESRD patients had reduced lymphocyte count (160±66±5µ/L vs. 249±95±2±µ/L; p<0.001) and B cell (CD19+) count (82.7±15.4µ/L vs. 177.6±7.8µ/L; p<0.001) compared to controls, whereas the percentages of CD8 cell subsets were not particularly affected, except for B1a subset which presented a significant increase (4.183% ± 0.710% vs. p<0.001), the absolute number of almost all subsets was significantly smaller in ESRD patients (CD19+ 81.3±60.4 µ/L vs. 81.3±60.4 µ/L; p=0.005, Naive:55.6±46.6 µ/L vs. 97.2±46.6 µ/L; p=0.004, Memory: 27.1±15.5 µ/L vs. 83.5±56.5 µ/L; p=0.01, CD19+Baff+ 58.1±42.7 µ/L vs. 117.9±59.8 µ/L; p=0.001). In 11 patients who had a follow-up 6 months after starting on renal replacement treatment no differences were found, apart from CD19+ IgM (74.7±4.7µ/L vs. post 66.9±14.7µ/L; p=0.014) and B1a percentage (3.02% ± 1.010% vs. p=0.038), which further decreased. Conclusions: Significant reduction was noticed in B cells in patients with ESRD on pre-dialysis stage, and in some of them further reduction was noticed in post-dialysis stage, and these changes may be implicated in clinical manifestations, such as frequent infections or impaired response to vaccination.
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150

P.A3.03.05
Clinical usefulness of autoantibodies to Myo-type phospholipase A2 receptor for diagnostic and monitoring disease activity in idiopathic membranous nephropathy

D. kheifiz-touhami, y. louinci, x. amoura, M. benhalima;
Mustapha baha teaching hospital, Algers, Algeria.

Objectives PL2AR is the major target antigen in adult idiopathic membranous nephropathy (IMN). This study aimed to assess these antibodies (Abs) prevalence and specificity in a cohort of IMN Algerian patients and to correlate this Abs with clinical parameters reflecting the disease activity. Patients and methods We measured anti-PL2AR Abs using an immuno-enzymatic assay in the serum of 40 patients with IMN, 09 with secondary MN and 10 with other forms of primary glomerular diseases. Anti-PL2AR Abs were correlated with proteinuria, serum albumin and serum creatinine in IMN patients. In 6 anti-PL2AR positive IMN patients, Abs levels were assessed at various stages of clinical disease and correlated with disease activity. Results Anti-PL2AR Abs were detected in 57,5% of IMN patients, but not in secondary MN or other forms of primary glomerular diseases. In 24 IMN patient, proteinuria was >3g/24h at the time Abs measurement. 23 (91,66%) of them were positive for PL2AR Abs, while in 14 patients, proteinuria was < 3g/24h. Among them, only 1 patient was positive for Abs. In IMN patients, Abs levels correlated positively with proteinuria and negatively with serum albumin. No correlation was found between Abs and serum creatinine. During the clinical course of the 6 anti-PL2AR positive patients, Abs levels correlated with clinical status, which were high at the acute phase disease and decreased during remission. Conclusions anti-PL2AR Abs is a sensitive and specific test for IMN. Abs levels correlate with clinical disease activity, there measurement may provide a tool for monitoring disease activity.

P.A3.03.06
Gene expression profile of tolerogenic dendritic cells differentiated with vitamin D3, dexamethasone and rapamycin

1Germs Trias i Pujol University Hospital and Research Institute, Campus Can Run, Badalona, Spain; 2Department of Statistics. University of Barcelona, Barcelona, Spain; 3Germs Trias i Pujol University Hospital and Research Institute, Barcelona, Spain; 4CICS-UBI-Centro de Investigación en Ciencias de la Salud, Covilhã, Portugal, 5CICS-UBI-Centro de Investigación en Ciencias de la Salud, Covilhã, Portugal; 6Centro de Investigación en Ciencias de la Salud, Covilhã, Portugal.

Background: Tolerogenic dendritic cell (tolDC)-based therapies have become promising approaches for the treatment of autoimmune diseases by their potential ability to restore immune tolerance in an antigen-specific manner. There is a broad variety of protocols to generate toIDC in vitro, being their differentiation in the presence of vitamin D3 (vitD3-tolDC), dexamethasone (dexa-tolDC) or rapamycin (rapa-tolDC) three of the most frequent. However, the characteristics of these cells are very heterogeneous, thus making the need to find common genetic pathways and biomarkers of high relevance. Objective: We compared the transcriptomic profile of vitD3-tolDC, dexa-tolDC and rapa-tolDC in order to find common induced pathways and biomarkers. Methods: Monocyte-derived dendritic cell stimulations of immature (iDC), mature (mDC), vitD3-tolDC, dexa-tolDC and rapa-tolDC from 5 healthy donors were generated, and a microarray analysis was performed (Affymetrix). Results: Normalized and filtered, and differentially expressed genes (DEG) were selected. A Gene Set Enrichment Analysis (GSEA) was performed to select common enriched pathways. Statistical analyses were performed using R software. Results: Common DEG could not be found for the three tolDC, although 14 genes (many of them immune-related) appeared up-regulated in at least one condition. GSEA revealed 11 common protein sets differentially expressed in tolDC. However, all of them were induced for vitD3-tolDC and dexa-tolDC, while down-regulated in rapa-tolDC. Conclusions: The analysis revealed that, despite not sharing potential common biomarkers, vitD3-tolDC and dexa-tolDC presented similar transcriptomic profiles, suggesting an induction of immune tolerance through common pathways, while rapa-tolDC seem to develop their function through different ones.

P.A3.03.07
Higher responsiveness of CLL cells to B-cell receptor stimulation is associated with reduced expression of inhibitory molecules of the NF-κB pathway

1Dept. of Immunology, Erasmus MC, Rotterdam, Netherlands; 2Dept. of Pulmonary Medicine, Erasmus MC, Rotterdam, Netherlands.

Background Chronic lymphocytic leukemia (CLL) is a heterogeneous disease based on both clinical and biological characteristics. We previously described (Muggen et al, Leukemia, 2015) differences in Ca2+ levels among CLL cases (both basal and upon B-cell receptor (BCR) stimulation). Such differences in BCR responsiveness could reflect a heterogeneity in CLL pathogenesis due to cell-intrinsic factors. Aim: To elucidate cell-intrinsic differences between BCR-unresponsive and BCR-responsive CLL patients. Methods: From 52 CLL cases, the BCR responsiveness was determined ex vivo based on Ca2+- influx upon α-IgM stimulation. Phosphorylation levels of various BCR-signaling molecules, as well as the expression of activation markers were assessed by flow cytometry. Transcription profiling of BCR-responsive (n=6) and BCR-unresponsive CLL cases (n=6) was performed by RNA sequencing. RQ-PCR was used to validate transcript level differences. Results: The increase in Ca2+ after α-IgM stimulation was age dependent by higher phosphorylation levels of PLCγ2, Akt and higher expression levels of CD21, CD80 and CD86. RNA sequencing revealed differences between the two groups, especially in expression of NF-κB pathway genes. RQ-PCR validation in additional CLL cases confirmed the lower expression of the critical NF-κB inhibitors NFKB1 (p=0.021) and NFKB2 (p=0.009) genes in BCR-responsive CLL. Likewise, expression of the potential NF-κB inhibitors NFKB1 (p=0.017) and NFKB2 (p=0.009) was reduced. Conclusion: From our data we conclude that BCR-responsive CLL cells have a more activated cell surface phenotype and reduced expression of components that are associated with inhibition of NF-κB signalling. This study illustrated that enhanced NF-κB activation is critical for the BCR responsiveness of CLL cells.

P.A3.03.08
Circulating Dendritic cells and monocyte subsets in multiple sclerosis patients

A. Monteiro1, C. Cruto2, P. Rosado3, L. Rosado3, M. Fonseca1, A. Paiva1;
1CICS-UBI-Centro de Investigación en Ciencias de la Salud, Covilhã, Portugal, 2Servicio Patología Clínica, Centro Hospitalar Covas da Beira, Covilhã, Portugal, 3Servicio Neurología, Centro Hospitalar Covas da Beira, Covilhã, Portugal.

Objectives: In multiple sclerosis (MS), immune cells play a crucial role in the pathogenesis of the disease. Some of the immune cells have been shown to be altered in MS patients. Activation markers can be used as a parameter of disease activity. Aim: To investigate the expression of activation markers in circulating monocytes and dendritic cells in multiple sclerosis patients. Methods: Peripheral blood mononuclear cells (PBMCs) from healthy subjects and patients with multiple sclerosis were stained with various markers and analyzed by flow cytometry. Results: The expression of activation markers in circulating monocytes and dendritic cells was increased in multiple sclerosis patients compared to healthy controls. Conclusion: The increased expression of activation markers in circulating monocytes and dendritic cells can be used as a parameter of disease activity in multiple sclerosis patients.

P.A3.03.09
Chronic Lymphocytic Leukemia: Increased IL-10 and STAT3 Levels in B cells

Ö. Özcan1, M. Gelmez2, S. Cınar3, G. Deniz1, M. Aktan1;
1Istanbul University Asia Sancar Institute of Experimental Medicine, Istanbul, Turkey, 2Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Istanbul, Turkey.

Chronic Lymphocytic Leukemia (CLL) is characterized by the accumulation of CD5+CD19+ B cells in the bone marrow and peripheral blood. Recent studies indicated that expression of IL-10, AID and miR-155 that are regulated by STAT3 are increased in CLL patients. CD5+CD19+ regulator B (Breg) cells secrete IL-10 and suppress the immune system. While the CLL cells show similar immunophenotypic properties to Breg cells, they are also thought to be functionally similar. In this study, levels of STAT3 and IL-10 in CLL patients were investigated. Peripheral blood samples obtained from patients (n: 24) and healthy subjects (n: 14). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient method. PBMCs (1x10^6 cell/ml) were cultured for 48 hours in the presence and absence of CpG (1 ug/ml) for IL-10 expression and for STAT3 expression cultured with and without PMA (1 ug/ml) for 6 min. IL-10 and STAT3 expressions were analysed using anti-CD5, anti-CD19, 5 monocytes and healthy subjects, in lymphocyte population increased IL-10, IL-10*CD19+ and STAT3*CD19+ cells were obtained (p<0.0001, p<0.0001 and p<0.0001, respectively). CD19+ B cells showed elevated IL-10 content in CLL patients (p<0.0001). Similarly, increased IL-10 levels (p<0.0001) were also found in CD5*CD19+ cells, whereas STAT3 levels were diminished (p=0.0074). These results suggest that for the levels of IL-10 and STAT3 in CLL patients, B cells are clearly different from normal B-lymphocytes might have a role in the biology of CLL.
In all lymphocyte progenitor cells' maturation process break down and proliferation become uncontrollable. Studies suggesting the use of NK cells capable of lysing tumor cells in the treatment of ALL. In this study, the response of NK cells to cytokines in B-ALL cases and the correlation with assessment of minimal residual disease (MRD) by flow cytometry according to International BFM Study Group protocol were investigated. CD7, CD10, CD19, CD34, CD45 cell surface expressions were detected in both B-ALL blast phenotypes. CD3, CD16 and CD56 cell surface expressions were detected in BM and peripheral blood (PB) samples of sALL. Mononuclear cells, isolated from PB and BM obtained from B-ALL patients at day of diagnosis, were stimulated with IL-2 for 24 hours and PMA+I0 for 5 hours. Intracellular IFN-y and IL-10 levels in NK cell subsets were determined by flow cytometry. PB CD3 CD56(+/-)CD16 and CD3 CD56(+/-)CD16 NK cell frequencies were found to be higher than those from BM. Although IL-10 content of CD3 CD56(+)CD16 and CD3 CD56(+/-)CD16 NK cell subsets were decreased, IFN-y content of both subsets of NK cells were diminished after stimulation both with IL-2 and PMA+I0. After treatment on day 15, IFN-y levels of CD3 CD56(+)CD16 NK cells were elevated by IL-2 stimulation only in patients detected as low risk. Our findings support that increased IFN-y secreting CD3 CD56(+/-)CD16 NK cell subset in FLR patients, after treatment of B-ALL according to the International BFM Study Group protocol, might have an indicator for the following of MRD-ALL at FLR patient.

The Role of Phagocytic Cells in Acute Respiratory Distress Syndrome

1Center for Pathophysiology, Infectionology and Immunology, MUW, Vienna, Austria, 2Department of Anesthesia, General Intensive Care and Pain Medicine, MUW, Vienna, Austria.

Acute respiratory distress syndrome (ARDS) remains a disputed clinical entity in terms of definition, pathophysiological understanding and therapeutic approach. Due to high heterogeneity, ARDS subgroups are best studied individually. Our focus lies on a) ARDS associated with pneumonia and b) ARDS in trauma patients. By means of multicolor flow cytometry, we provide a comprehensive analysis of surface makers in sputum, tracheal aspirate and bronchoalveolar lavage (BAL) cells. Sputum is obtained from lung-healthy volunteers, whereas tracheal aspirate and BAL are collected from lung-healthy intubated patients, as well as from patients diagnosed with ARDS. By comparing samples obtained from lung-healthy controls to those of ARDS patients, we aim to pinpoint cell populations that undergo significant changes in pathological states, with a focus on phagocytic cells and monocytes/macrophages.

Conclusions: Our results confirm the hypothesis that ARDS in patients with pneumonia is caused by a local inflammatory response, whereas ARDS in trauma patients is triggered by injury of the pleura. Once we recently identify the populations most relevant for the studied condition, we proceed to performing mRNA-Sequencing of the fluorescence-activated cell-sorted phagocytes. By characterizing the expression patterns in ARDS patients depending on the underlying pathology and by discriminating these from those induced by mechanical ventilation, this study will provide a basis for identifying individual risk factors and biomarkers of lung disease on the intensive care unit.

Heavy/light chain (HLC) ratio measurements in intact immunoglobulin multiple myeloma (IIMM) patients-a single center experience

1Sanquin, Amsterdam, Netherlands, 2Leiden University Medical Center, Leiden, Netherlands.

Here we describe how surface plasma resonance bioraster array can be utilized to determine and thereby to characterize the biological activity of platelet-bound antibodies. Current methods applying anti-human IgG using whole platelets measure both antibodies targeting the platelets and those bound to platelet FcR. We hypothesized that this can be overcome using a human FcR for detection of whole Fab-binders antibodies targeting platelets. At the same time this can provide an evaluation reflecting the biological activity of the platelet-bound antibodies. This would improve the diagnostic work-up both in fetal and neonatal alloimmune thrombocytopenia (FNAIT) and in immune thrombocytopenia (ITP).

To test our hypothesis we compared the binding of chloroquine-treated platelets (removing HLA antigens), opsonized with serum samples from 166 women with anti-HPA-1a antibodies causing FNAIT, to anti-IgG and to FcR coupled onto the bioraster surface. Our results show that compared to anti-IgG, the binding by FcR of these opsonized platelets shows a stronger correlation (Pearson's r=0.56 and 0.67, respectively) to the anti-HPA-1a antibody levels measured by monoclonal-antibody immobilization of platelet antigens (MAIPA). In addition, we have combined this technique with anti-complement (C3) and anti-IgA and anti-IgM and show that these features can also be evaluated simultaneously and directly on primary material from patients with ITP (n=50). These methods show that SPR technology can be applied to get reliable clinically relevant data that go beyond what is possible with current diagnostic techniques.
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P.A3.03.15
Incidence of CMV infection in childhood

N. Zators, P. Christodoulou, E. Tatsina, N. Varsamis, A. Zotou, F. Adam, G. Katagios, L. Papageorgiou, M. Gianniki, N. Tsifetaki

1 General Hospital of Ioannina, Greece, Ioannina, Greece, Papageorgiou Hospital, Thessaloniki, Greece, 2 University Hospital of Ioannina, Ioannina, Greece, 3 Agia Sofia Hospital, Athens, Greece.

Aim: To determine the incidence of CMV infection in children during a two-year period (2016-2017) Method: 980 children of 1-14 years of age who presented to the outpatient department of the hospital with symptoms of the disease or hospitalized from January 2016 until December 2017, were the material of the study. The samples were tested for IgG and IgM antibodies against CMV by ELISA (AxSYM,Abbott). The diagnosis was set either by detecting both IgG and IgM antibodies or by detecting a fourfold elevation of the titre of IgG antibodies in the second serum sample. Results: No antibodies were detected in 520 (53.0%) out of 980 children that were tested for CMV antibodies, while 460 (47.0%) were positive. 232 (50.4%) were suffering from CMV infection.

139 (59.5%) out of 232 were initially positive only for IgM antibodies, 73 (31.5%) were positive for IgG and IgM antibodies while 20 (8.6%) were positive only for IgG antibodies and presented a fourfold increase of their titre in the second sample. The remaining 229 (49.6%) presented a lower titre of IgG antibodies in both serum samples. There was no significant difference in two sexes. Epidemiological factors, such as social-economic status, follow-up in daily care centers and cohabitation in large numbers don’t correlate with CMV sero-positivity. Conclusion: The study revealed that CMV infection is not rare. Due to severe complications in infancy, it is important to diagnose the disease quickly and efficiently so that proper personalized care is delivered.

P.A3.03.16
Estimation of two serological markers in the diagnosis of rheumatoid arthritis

N. Zators, P. Christodoulou, E. Tatsina, N. Varsamis, A. Zotou, G. Katagios, F. Adam, A. Pourmou, M. Gianniki, N. Tsifetaki

1 General Hospital of Ioannina, Greece, Ioannina, Greece, Papageorgiou Hospital, Thessaloniki, Greece, 2 University Hospital of Ioannina, Ioannina, Greece, 3 Agia Sofia Hospital, Athens, Greece.

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology. Aim: To determine the frequency of antibodies against synthetic circular citrullinyl peptides (anti-CCP) and to compare them with rheumatoid factor (RF) in a group of patients with rheumatoid arthritis symptoms. Material-METHOD: 74 sera that were referred to the Laboratory with symptoms of mild to very severe rheumatoid arthritis were tested for the presence of anti-CCP and IgM RF antibodies. Control of the first was performed with enzyme immunoassay (ELISA) or latex agglutination (LA) and of the second with the method of neubometry. Results: In 44 out of 74 patients the titles of both antibodies ranged in normal levels. Of the others, 18 had elevated titles of both antibodies while IgM were detected in 12. The 44 patients with negative antibody titles and 12 with only RF antibodies positive, showed mild and non-specific symptoms of the disease whereas the 18 patients with elevated anti-CCP and IgM RF titles had clinical picture consistent with rheumatoid arthritis. Conclusions: Anti-CCP antibodies are an important and specific indicator in the diagnosis of rheumatoid arthritis. Rheumatoid factor, although a sensitive marker for RA, lags behind in its specificity because it is detected in small percentages in other autoimmune diseases.

P.A3.03.17
Incidence of antibodies against Toxoplasma gondii, Rubella virus, Cytomegalovirus and herpes simplex virus in Greek and foreign women

N. Zators, E. Tatsina, L. Papageorgiou, E. Chrysohontomou, P. Christodoulou, A. Zotou, N. Varsamis, A. Pourmou

1 General Hospital of Ioannina, Greece, Ioannina, Greece, Papageorgiou Hospital, Thessaloniki, Greece, 2 University Hospital of Ioannina, Ioannina, Greece.

Aim: To determine the incidence of infections caused by Toxoplasmagondii, Rubella Virus, Cytomegalovirus (CMV) and Herpes Virus Simplex (HSV) in Greek and foreign women, presenting in a tertiary hospital of North-Western Greece. Material and Methods: 382 women (278 Greeks and 104 Foreigners leaving in Greece) were tested for antibodies against T. gondii, Rubella Virus, HSV during one year (January 2017 to December 2017). 327 were tested for antibodies against T. gondii, 238 were tested for antibodies against Rubella Virus, 302 were tested for anti-CMV antibodies and 124 for anti-HSV antibodies. 92 of the women were tested for antibodies against all of the pathogens (TORCH). An enzyme-linked immunosassay was employed for the detection of all antibodies. Results: It is important to note that there was a statistically significant difference in the seropositivity against anti-Toxoplasma IgG and anti-CMV IgG antibodies in foreign women in comparison to Greek women (19.8% and 10.2%, 88.6% and 71.8%, respectively). There was no difference as far as positivity to antibodies against T. gondii, Rubella Virus, CMV and HSV detected in women, both Greek and Foreign, makes the evaluation of the population inevitable. In this way infection prevention and control can be accomplished.

P.A3.03.18
Rapid Target Identification for T-Cell Immune Responses with SpotMix™ Peptide Pools

U. Reimer, M. Eckey, P. Holena, T. A. Teck, J. Zerweck, V. Knaute, F. Kern

JPT Peptide Technologies, Berlin, Germany.

Introduction: Vaccinations inducing or boosting T-cell immunity are successfully used in the infectious diseases and cancer fields. Suitable vaccine target proteins must contain T-cell stimulating peptides providing good epitopes. However, to date, algorithms for T-cell epitope prediction cannot reliably identify the most promising target proteins. Protein-sequencing, overlapping pools of conventional synthetic peptides (PepMix™) representing all possible stimulating peptides in a target protein and are, therefore, ideally suited for this purpose. However, they are too costly for testing multiple potential target proteins.

Methods: Based on a method for the highly parallel synthesis of multiple peptides in low quantities, referred to as Spot synthesis, we developed a novel protocol for synthesizing peptide pools and SpotMix™ pools demonstrated similar performance of these preparations.

Conclusions: SpotMix™ peptide pools will significantly facilitate T-cell protein target discovery by permitting the synthesis of protein-spanning, overlapping peptide pools for many potential target antigens in parallel. These can be tested experimentally and the most promising target proteins can be selected for further investigation. Since Spot peptides are unpurified and produced in small quantities (approx. 10nmol) they are unsuitable for T-cell expansions or other therapeutic applications, however, represent ideal tools for T-cell target discovery.

P.A3.03.19
Fine characterization of healthy Conjuvantia: Main differences when comparing IELs and peripheral blood lymphocyte subsets

J. Zos, C. Alfredo

Universidad de Valladolid, Valladolid, Spain.

Introduction: As occurs in other mucosal tissues —for example gut, bronchi and nose—, ocular mucosa holds a conjunctiva-associated lymphoid tissue (CALT). It is well known that MALT (Mucosa Associated Lymphoid Tissue) has morphological and functional variations across tissues. Therefore, a thorough analysis of lymphoid populations might render useful information on ocular surface conditions. Objectives: The aim of this study is to improve the knowledge of human immune system within the conjunctiva in different ocular surface conditions. Material and methods: Twenty-five healthy volunteers were recruited. Peripheral blood lymphocytes were obtained by venipuncture while intraepithelial lymphocytes (IELs) from conjunctival mucosa were obtained by brush cytology. Major and fine subsets were characterized by flow cytometry. Memory, naive, i&ε T cells, CD8+ (Tc, NKT subtypes), CD4+ (Th0, Th1, Th2, Th17, Th1/Th17, Th22 and Treg subsets), B cells (B1 and B2) and NK cells —regulatory and cytotoxic— subsets were analyzed in both conjunctival mucosa and peripheral blood. Results: Age and sex seemed to determine few differences in some lymphocyte subsets: Th1 cells might be age-influenced whereas Th22 might be sex-influenced. As expected, no strong correlations between peripheral and conjunctival lymphocytes were found. Conjuvantia T cells seemed to be mainly CD8+ and TCRβδ+, while they were only a minor population in peripheral blood. Memory CD4+ T cells, NKT, B1, Thgs and regulatory NK cells had higher values in conjunctiva. Conclusions: Some well-known differences (increased TCRβδ cells) whereas others are apparently new in our knowledge (increased B1, NKT, Tregs and regulatory NK lymphocytes) were found. These features provide an extra effect and regulatory function to the conjunctiva.
POSTER PRESENTATIONS

PA3.03.20
Cell-free tumoral DNA (ctDNA) based EGFR mutation analysis in a cohort of Indian non-small cell lung carcinoma (NSCLC) patients.
S. Verma Kumar, R. Katara, V. Kumar, J. Jandialp, S. Panjdey, S. Sharma, L. Kini; CORE Diagnostics, Gurugram, Haryana 122016, India., Gurugram, India.

Patients with lung cancer show poor prognosis, with a five-year survival rate of about 17.8%. Epidermal Growth Factor Receptor (EGFR) mutation analysis is critical for patient selection for targeted tyrosine kinase inhibitor (TKI) therapy, and immunotherapy. Mutations such as exon 19 deletions and L858R point mutation confer sensitivity to EGFR TKIs; while mutations such as T790M confer decreased sensitivity to first- and second-generation EGFR TKIs. For this study, a total population comprised of 627 (male: female ratio of 1.18:1) patients with primary NSCLC. The patients ranged in clinical presentation from Stages I to IV of lung cancer, and most had previously tested positive in EGFR mutation analysis on tissue biopsy. The ctDNA was tested for “hotspot mutations” in EGFR c.2573T>G (L858R); EGFR Deletion E746-A750; and EGFR c.2369C>T (T790M) using ddPCR.
We report a 45.15% positivity for EGFR mutations in this cohort of NSCLC patients. Further, 26.63% tested positive for Delelton E746-A750; 21.37% for T790M; and 13.87% for L858R. We also noted 16% cases showed concordance activity for Deletion E746-A750 and T790M, and 4.62% for L858R. As the sensitivity of ctDNA based EGFR testing ranged from 71-74% (as against tissue-based mutation analysis), we recommend testing all negative cases by a repeat tissue biopsy. We are currently carrying out a retrospective correlation of these test results with patients’ clinical data, especially with reference to disease progression.

PA3.03.21
Distinct cytokine patterns may regulate the severity of perinatal asphyxia
A. Bajkon, L. Berta, C. Orban, G. Tooldi; Semmelweis University, Budapest, Hungary.

Neuroinflammation following perinatal asphyxia may have dual aspects being a hindrance, but also a necessity in the recovery of the CNS. We aimed to assess intracellular cytokine levels of T lymphocytes and plasma cytokine levels in moderate and severe asphyxia in order to identify factors that may influence patient outcome. We analyzed data of neonates with moderate (n = 17) and a severe (n = 11) asphyxia. Grouping was based on neuroradiological and aEEG characteristics. Blood samples were collected at 6 h, at 24 h, 72 h, 1 week and 1 month of life. Blood samples were stimulated for 6 h, then intracellular cytokine levels were determined using flow cytometry. Cytokine plasma levels were measured using Bioplex immunoassays. The prevalence of IL-1β and CD4 cells was higher in severe asphyxia at 6 h and the prevalence of CD4+ IL-1β and CD4+ IL-6 cells appears to be able to predict severity of TNF-α as an important in the severe group. Plasma IL-6 levels were higher at 1 wk in the severe group and decreased by 1 mo in the moderate group. Intracellular TGF-β levels were increased from 24 h onwards in the moderate group only. IL-1β and IL-6 appear to play a key role in the early events of the inflammatory response, while TNF-α seems to be responsible for prolonged neuroinflammation, potentially contributing to a worse outcome. TGF-β has a compensatory role in decreasing inflammation.

PA3.03.22
Immunobiogram as a diagnostic assay for detection of resistance to immunomodulatory treatment in patients with chronic inflammatory diseases.

Introduction: The immune system is very complex, dynamic and with self-learning capacity. The response of patients with chronic inflammatory diseases treated with immunomodulatory drugs can change over time.
Resistance arises during immunomodulator treatment and is particularly problematic during long-term treatment regimes. Nowadays, there are no biomarkers or pharmacovigilance tools to monitor drug resistance development.
Methods: Biohope has designed the immunobiogram to be a “final point” immunoassay using a pharmacodynamic approach. We studied a cohort of transplant recipients treated with different immunosuppressants. The Immunobiogram allows to stablilize an individualized pattern of sensitivity/resistance for each patient.
Results: We verified the existence of different patterns of sensitivity/resistance as response to immunomodulator sensitive treatment in 70 patients. We detected that patients who had not been treated with a specific immunosuppressant are more sensitive to such treatment than patients who has been treated previously. Moreover, we detected the existence of a correlation between the dose supplied of a certain drug during the treatment and the decrease of the sensitivity. This decrease dose-dependent sensitivity is determined at least in part by the establishment of resistance to drug, which entails to an increase in the ID50 (half-maximal inhibitory dose) necessary for the maintenance of immunosuppression.
Conclusions: When evaluating the immunological status of the patient treated with immunosuppressants, it should be considered two antagonistic variables: the loss of synergistic activation networks of the immune system due to drug effect and the generation of molecular mechanisms of resistance to treatment. The Immunobiogram will allow us monitor appearance, or not, of these resistances.

PA3.04.04 Immunomonitoring and biomarkers - Part 4

PA3.04.01
A study of hs-CRPas cardiovascular risk marker in pre-hypertension
S. Dogo; School of Medical Sciences and Research, Greater Noida, India.

Background: Hypertension has turned into a leading cause of non-communicable disease associated with mortality and morbidity in both developing as well as developed world. The concept of pre-hypertension, defined as a systolic blood pressure of 120-139 mmHg and/or a diastolic blood pressure of 80-89 mmHg was introduced as the new guideline for the management of blood pressure. Hs-CRP has been studied extensively as marker of cardiovascular risk, however its role as a marker of cardiovascular risk in pre-hypertension is not well defined. The aim of this study was to explore the role hs-CRP cardiovascular risk marker in patients of pre-hypertension.
Methods: 50 adult patients, above 19 years of age, with diagnosed pre-hypertension and 50 age and sex matched healthy controls were studied in a tertiary health care center in Uttar Pradesh India, over a period of 6 months. The serum levels of hs-CRP was measured by ELISA and routine lipid profile was measured by automated analyzer. Framingham Risk scoring was also done for all the patients. Data is presented as Mean±SD. and relationships were determined by Pearson correlations.
Results: The mean age of the patients was 51±5.7 years (72% men, 28%women). The mean serum hs-CRP levels [5.40±2.51 mg/l] for pre-hypertension were significantly higher than in controls [0.9±1.76 mg/l] [p<0.001]. Framingham Risk score was higher for patients with pre-hypertension than controls. Higher hs-CRP values correlated with higher Framingham risk score.
Conclusion: Our results suggest that hs-CRP is a Marker of Cardiovascular Risk in patients of pre-hypertension.

PA3.04.02
Serum uromodulin, a new biomarker of renal function?
C. Esteve Cois1,2; F. Geroned Torres, M. Navarro Diaz; A. Soriano Martínez; J. Aro del Rey; E. Martínez Cáceres1,2; B. Quiroz Sánchez1.
1Immunology Department Hospital Universitari Germans Trias i Pujol, Badalona, Spain.; 2Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Bellaterra, Spain.; 3Nephrology Department Hospital Universitari Germans Trias i Pujol, Badalona, Spain.

Membranous Glomerulonephritis (MGN) and IgAN are the leading forms of primary membranous glomerulonephritis. MGN is caused by autoantibodies against Phospholipase A2 receptor, and IgAN by partially deagglutinated IgA1 (Dd-IgA1) that induces the generation of immunocomplexes of soluble IgA receptor (CD89) and IgA1. The glycoprotein uromodulin is synthesized exclusively in the ascending limb of loop of Henle. A decrease in serum values is a sensitive marker of low renal function. We aim to explore serum uromodulin as a biomarker of renal function, renal glomerular filtration (eGFR) and proteinuria. A retrospective study of 46 MGN, 22 IgAN patients diagnosed by renal biopsy and 9 Healthy Subjects (HS) was performed. Clinical and pathological features were collected and analyzed according to serum uromodulin levels. Analysis of serum uromodulin was performed with uromodulin-ELISA kit (Euroimmun®).
MGN and IgAN patients had lower levels of serum uromodulin than HD (MGN: 131 ± 74.31; IgAN: 91.79 ± 57.12; HS 224.9 ± 74.70 ng/mL). There were no differences between uromodulin levels and patients age or gender. We stratified the patients according to histopathological features of renal biopsy. MGN patients with positive biopsy for IgG4 deposits, showed a correlation between serum uromodulin-eGFR (p<0.0007, r=0.62) and serum uromodulin-creatinine (p=0.0565, r=0.38). The same results were observed in those IgAN patients with more severe renal biopsy (p<0.0037, r=0.76; p<0.0002, r=0.87), respectively. The inverse correlation observed between uromodulin levels and severity of renal biopsy suggests that uromodulin might be a prognostic biomarker of renal function, especially in IgAN patients.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 153
Therapy response varies in multiple sclerosis (MS) patients and still cannot be predicted. Interferon (IFN)-β, a first-line drug for relapsing-remitting (RR) MS is not efficient in up to 30% of RRMS patients. Therefore, identification of biomarkers predictive of therapy response is of utmost importance. The current study aimed to evaluate the effect of dimethyl fumarate (DMF, a well-tolerated oral compound) on T cell metabolism in RRMS patients treated with IFN-β and to relate these changes to the clinical outcome and potential adverse effects.

**Material and Methods:** Longitudinal prospective study of peripheral blood T, B, NK, monocyte and DC subpopulations using multiparametric flow cytometry in whole blood from 12 RRMS patients under DMF treatment at baseline and after 1, 3, 6 and 12 months of follow up.

**Results:** DMF reduces the number of clinical relapses in MS patients, and is associated with the effect of DMF depleting effector cells. The study evidenced a selective reduction of T effector, T central and B memory cells, and an increase in regulatory and memory T cells. Concurrently, immune monitoring over several leukocyte subsets showed a metabolic switch in favor of IFN-β treatment response.

**Conclusions:** DMF may be a useful tool to predict the clinical outcome and adverse effects of treatment in MS patients.
**POSTER PRESENTATIONS**

**P.A3.04.11**

**Determination of monocytes, dendritic cells, NK cells and T regulatory cells in patients with type 1 diabetes and healthy children**

A. Oroz, R. Urbá
University of Tartu, Tartu, Estonia.

**Introduction:** Type 1 diabetes (T1D) is an autoimmune disease characterized by immune-mediated destruction of the insulin-producing β-cells in pancreatic islets. Altered numbers of innate immune cells such as dendritic cells (DC), natural killer (NK) cells and monocytes as well as adaptive immune cells such as T regulatory (Treg) cells have been demonstrated in previous studies. The aim of this ongoing study is to compare the numbers of aforementioned cell populations in children with T1D and healthy controls.

**Material and Methods:** Whole blood was collected from patients with recent onset of T1D and healthy children. Relative numbers of DC, plasmacytoid DC, myeloid DC, monocytes, NK cells, Tregs and their subpopulations were determined based on differential expression of HLA-DR, CD16, CD56, CD14, CD123, CD11c, CD4, CD25, CD127 and CD194. For calculating the absolute cell counts BD TRUCOUNT Beads were used. The data was analyzed according to the COST-ENTIRE HIP-C version 3.3 protocol and immunophenotyping of whole blood was performed on the BD LSFortessa™. Mann-Whitney U test was employed to assess the difference in study groups.

**Results:** Our studies revealed significantly different differences in dendritic cells subsets between patients with T1D and healthy controls. **Conclusion:** Our results confirm the suitability of HIP-C 3.3 protocol for testing peripheral blood innate immunity and Treg cell subpopulations; and support the possible involvement of some of these subsets in T1D pathogenesis.

**P.A3.04.12**

**A/Professor, PhD**

D. Jain, M. O’Malley, K. M. Pouluss
Experimental Medical Science, Lund, Sweden.

Tapsin edits HLA-I molecules and hence plays a crucial role in immunological surveillance. Our novel data shows that recombiant tapsin dissolves abberant conformations of HLA-I when added to the cell surface. During inflammation and in tumours pH is decreased. To study the effect on tapsin secondary structure at different pH and temperature we here used Synchrotron Radiation Circular Dichroism (SRCID) spectroscopy and Small Angle X-ray Scattering (SAXS) to study the dynamic solution structure of tapsin under physiological conditions. Contrary to crystal structure data, which was performed at room temperature, we found that tapsin undergoes structural transition and acquires a characteristic fold at physiological temperature i.e. at 37°C. Interestingly, decreased pH also resulted in the same conformational change of structure. These results show a conformational change in tapsin at low pH as well as at physiological temperature, which is highly relevant to consider when e.g. developing new peptide based vaccines or studying HLA-I antigen presentation at physiological temperature and in inflamed tissue with lower pH.

**P.A3.04.13**

**Relationship between serum protein pattern and disease activity in patients with systemic sclerosis**

Faculty of Medicine and Dentistry, Palacký University and Hospital Olomouc, Olomouc, Czech Republic.

**Introduction:** Systemic sclerosis (SSc) is a complex autoimmune connective tissue disorder with varying manifestations and clinical outcomes. There is still limited information of serum biomarkers suitable for monitoring efficacy of disease specific therapy.

**Methods:** We investigated the serum levels of 92 inflammation-related proteins in 52 Czech patients with SSc and 24 age/gender-matched healthy control subjects using a highly sensitive innovative multiplex PEA (Olink Bioscience, Sweden). Subgroups were formed based on the disease activity (non-active SSc, n=14; active SSc, n=38), where revised EUSTAR activity index of >2.25 was taken as active SSc. Statistics were performed using GenEx (Sweden).

**Results:** Top-ranked proteins distinguishing SSc and healthy controls (P<0.00001) were TNFα, interleukin 1α, IL-10, IL-6, IL-7, IL-8, IL-12, IL-18, IL-23, IL-23, IL-24, IL-31. When the amount of cells was very low total RNA was isolated and PCR was performed. When comparing SSc patients with active and non-active disease, upregulation of IL-6, CCL7, IL-10, CCL11, TNFβ, IL-12β, and sTNFβ was observed to be associated with disease activity (P<0.05). Moreover, serum levels of IL-6, CCL7, IL-10, CCL11, and downregulation of TNFβ was observed to be associated with disease activity (P<0.05). In contrast, IL-12β and IL-17A positively correlated (P<0.00001 and P<0.0005, respectively) and TNFβ negatively correlated (P<0.001) with disease activity.

**Conclusions:** This study nominated novel serum markers IL-6, CCL7, IL-10, CCL11, sTNFβ, for evaluation of the disease activity of patients with SSc. Larger cohorts and multivariate analysis will be needed to prove their usefulness as biomarkers for active SSc.

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**P.A3.04.14**

**Phagocyte metabolic profile in different models of Parkinson's disease in rats**

1Institute of Biology and medicine, Kyiv, Ukraine, 2National Cancer Institute, Kyiv, Ukraine.

**Introduction:** The local inflammation in CNS during Parkinson's disease (PD) is associated with phenotypic and functional changes not only in local microglia but also in peripheral phagocytes. The aim of this study was to compare the functional state of phagocytes from different locations in rats with MPTP-induced and 6-OHDA-induced PD. **Methods:** PD in rats was induced with subcutaneous injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or unilateral stereotaxic injection of 6-Hydroxydopamine (6-OHDA) into the striatum. Reactive oxygen species (ROS) generation and phagocytosis activity, as well as CD14, CD68, CCL80/86 and C206 expression by microglia, circulating phagocytes and peripheral macrophages were evaluated by flow cytometry. NO production and arginase activity of the cells were examined in colorimetric assays. Results: MPTP-induced PD in rats was associated with increase in highly phagocytic CD14+CD68+ microglia fraction. CD14+CD68+ peripheral macrophages were characterised by increased NO production. Circulating phagocytes showed upregulated C6960, while their ROS production and phagocytosis were decreased. In rats with 6-OHDA-induced PD, sharply decreased phagocytosis along with increased ROS and NO production by microglia were detected. Decreased CD14 and CCL80/86 expression, along with high CD206 expression were observed on microglia of 6-OHDA-induced PD. High phagocytic neural damage led to microglial activation. Circulating phagocytes and peripheral macrophages showed pro-inflammatory activation. **Conclusion:** Inflammatory process in CNS of rats with PD was characterised by their microglia strong activation, more pronounced in case of 6-OHDA-induced PD.

**P.A3.04.15**

**Immunomonitoring of Treg heterogeneity in Multiple Sclerosis**

1Laboratory of Neuroimmunology, Fondazione Santa Lucia, Rome, Italy, 2Institute of Experimental Oncology and Endocrinology, National Research Council (IEOS-CNR), Immunology Lab, Naples, Italy, 3Department of Neurosciences, San Camillo Forlanini Hospital, Rome, Italy, 4Department of Neurology and Psychiatry, Sapienza University of Rome, Rome, Italy, 5Laboratory of Neuroimmunology, Fondazione Santa Lucia, Rome, Italy, 6Department of Molecular Medicine and Biotechnologies, University of Naples “Federico II”, Naples, Italy.

**Introduction:** Regulatory T cells (Treg) are a fundamental component for immune regulation and homeostasis. In humans, Tregs are a heterogeneous population for gene expression, phenotype, and suppressive functions. While there is a general consensus regarding the surface markers required for their identification, several studies have identified distinct subsets within this cell population based on the evidence that several FoxP3 isoforms exist. The two most relevant FoxP3 isoforms are the full length isoform (FoxP3FL) and the isoform lacking exon 2. Since FoxP3 regulates the development and function of Treg cells, different FoxP3 isoforms lead to different Treg cells.

**Material and Methods:** Fresh PBMC (ex-vivo) from MS patients and healthy controls (HC) were stained to define the phenotype of Treg cells, using antibody clones that detect different FoxP3 isoforms. Total lymph cells were used to perform Western Blots. When the amount of cells was very low total RNA was isolated and PCR was performed.

**Results:** We find that Treg cells from MS patients preferentially express the FoxP3 isoform lacking exon 2. This isoform confers reduced suppressive abilities. We also find that in patients T reg cells express markers of cellular exhaustion. These findings may explain the the less effective immune regulation found in patients with autoimmune disease.

**Conclusion:** This study underlines the importance of studying T regulatory cells taking in consideration the existence of FoxP3 isoforms which identify cells with potentially diverse suppressive abilities and which may be differently expressed in health and disease.

**Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands** 155
The evaluation of both a negative and a positive result should be done with great care.

Of the 29 marginal activity sera, 6 showed anti-ENA (+) and one sample with a known SLE history showed DNA (anti-dsDNA) and anti-extrapolated core antigens (ENA) in patients both internals and externals.

Material-Method: A total of 383 patients (126 internal and 257 external) were assessed.

The levels of IL-6 (p=0.038), IL-7 (p=0.028) and MMP-10 (p=0.038) in UC patients during remission, dropped significantly to the level of HC (p<0.01) after follow-up. Patients who did not respond on corticosteroids (n=5) had higher levels of IL-6 (p=0.001) and IL-13 (p=0.005) at baseline; non-responders on anti-TNF treatment (n=3) had higher IL-10 (p=0.006) levels. The levels of IL-6 (p=0.001) and IL-10 (p=0.006) were significantly decreased in patients with severe disease.

Among ten clock genes that were examined, REVERBα, PER1, and PER3 showed altered rhythmic patterns. The levels of IL-6 (p=0.038), IL-7 (p=0.028) and MMP-10 (p=0.038) in UC patients during remission were significantly below the level of HC (p<0.01) after follow-up. Patients who did not respond on corticosteroids (n=5) had higher levels of IL-6 (p=0.001) and IL-13 (p=0.005) at baseline; non-responders on anti-TNF treatment (n=3) had higher IL-10 (p=0.006) levels. The levels of IL-6 (p=0.001) and IL-10 (p=0.006) were significantly decreased in patients with severe disease.

Markers of angiogenesis hold promise as predictors of disease outcome at baseline and during treatment. Validation of this angiogenesis signature in independent cohorts is required. Whether patients in treatment-free remission may still suffer from smouldering vessel wall inflammation needs further investigation.

Circadian rhythm of immune cells in healthy individuals and patients with rheumatoid arthritis

Circadian rhythms in the tongue, nasal cavity, small intestine and brain promotes the body’s intake and absorption of lipids, and increases the risk factors of metabolic diseases. Serum soluble CD36 is found as a component of circulating microparticles (MPs) and may be as a predictor for atherosclerotic disease. Inhibition of CD36 expression or interference with its associated signaling pathways can significantly alleviate the severity of atherosclerosis. In addition, the highly expression of CD36 in the tongue, nasal cavity, small intestine and brain promotes the body’s intake and absorption of lipids, and increases the risk factors of metabolic diseases. Serum soluble CD36 is found as a component of circulating microparticles (MPs) and may be as a predictor for atherosclerotic disease.
POSTER PRESENTATIONS

P.A3.04.23
Electrochemical biosensing of cancer exosomes in human serum based on magnetic separation
S. Lima do Moura1, M. Pivdian1, M. Martí2
1Grup Sensors i Biosensors. Dpt. Química. Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain, 2Institut de Biotecnologia i Biomedicina, Bellaterra (Barcelona), Spain.

The identification of novel biomarkers represents a worldwide challenge not only for the improvement of early diagnostics, but also for patient monitoring and for the evaluation of the efficiency of a therapeutic strategy. Exosomes are nano-sized and cup-shaped vesicles, which are currently under intensive study as potential diagnostic biomarkers for many health disorders, including cancer. Therefore, this is a growing need for sensitive methods capable of accurately and specifically determining the concentration of exosomes. This work presents the study of different receptor by flow cytometry as well as the design of a quantitative and rapid method for total exosome counting based on magneto-actuated platforms with electrochemical readout. Two different strategies were explored for the magnetic separation of exosomes. Briefly, based on i) the direct covalent immobilization on toxin-activated magnetic particles or, instead, ii) immunomagnetic separation based on different receptors. The magneto electrochemical bio sensor for the exosomes counting was successfully achieved in human serum. This proof-of-concept device represents a rapid, cost-effective, and high-sensitivity detection of exosomes and can be potentially established as promising approach for cancer diagnostics based on liquid biopsy.

P.A3.04.24
Magnetic-actuated rapid test for the detection of circulating tumor cells
A. Pallerás1, S. Lima do Moura1, M. Mesas1, M. Martí1, M. Pivdian1
1Grup Sensors i Biosensors. Dpt. Química. Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain, 2Institut de Biotecnologia i Biomedicina, Bellaterra (Barcelona), Spain.

According to WHO, breast cancer is the top cancer in women both in the developed and the developing world, and the number of new cases is expected to rise by about 70% over the next two decades. The early and accurate detection of breast cancer as well as the risk of metastasis in small healthcare centers remains as the cornerstone of breast cancer control. This work is intended to contribute in the development of Rapid Diagnostic Test (RDTs) for cancer diagnosis at point-of-care in low resource settings, taking breast cancer circulating tumor cells from MCF7 systemic blood as model. Two different strategies were designed: a magneto-actuated immunosensor for the quantification of the breast cancer cells and a magnetic genosensor for the detection of the PCR-amplified genetic material from the cells. For that purpose, different commercial antibodies against specific epitopes of the cellular membrane were firstly studied by flow cytometry and confocal microscopy. Such antibodies were then covalently immobilized on magnetic particles to capture the tumor cells by immunomagnetic separation for the preconcentration of the cells from complex samples and immunosensing with an specific antibody. The magnetic genosensing approach is based on a double-tagger RT-PCR amplification of the transcripts from the cells and the quantification of the amplicon by amperometry technique or visual readout based on lateral flow. Finally, the results of these strategies are compared in terms of the analytical performance, showing promising features for being used as RDTs.

P.A3.05 Immunomonitoring and biomarkers - Part 5

P.A3.05.01
Innate immune recovery predicts CD4+ T-cell reconstitution after hematopoietic cell transplantation
C. de Koning1, J. Langenhorst2, C. van Kesteren3, C. A. Lindemans3, A. Huijtema3, S. Nierkens3, J. J. Boelens1,2
1University Medical Centre Utrecht, Utrecht, Netherlands, 2Wilhelmina Children's Hospital, Utrecht, Netherlands.

Innate immune cells are the first to recover after allogeneic hematopoietic cell transplantation (HCT). Nevertheless, reports of innate immune cell recovery and their relation to adaptive recovery after HCT, are largely lacking. Especially predicting CD4+ T-cell reconstitution is of clinical interest, as this parameter directly associates with survival chances after HCT.

We developed a multivariate, combined non-linear models effect model for monocytes, neutrophils and NK-cell recovery after transplantation. We evaluated whether innate recovery relates to CD4+ T-cell reconstitution probability, and investigated differences between innate recovery after cord blood transplantation (CBT) and bone marrow transplantation (BMT).

205 Patients, undergoing a first HCT (76 BMT, 129 CBT) between 2007-2016, were included. The median age was 7.3 years (range 0.16-23). Innate recovery was highly associated with CD4+ T-cell reconstitution probability (p<0.001) in multivariate analysis correcting for covariates. Monocyte (p=0.001), neutrophil (p=0.001), and NK-cell (p=0.001) recovery reached higher levels during the first 200 days after HCT compared to BMT. The higher innate recovery after CBT may be explained by increased proliferation capacity (measured by Ki-67 expression) of innate cells in CB-grafts compared to BM-grafts (p=0.041), and of innate cells in vivo after CBT compared to BMT (p=0.048). At an individual level, patients with increased innate recovery after either CBT or BMT had received grafts with higher proliferating innate cells (CB: p=0.004, BM: p=0.01, respectively).

Our findings implicate the use of early innate immune monitoring to predict the chance of CD4+ T-cell reconstitution after HCT, with respect to higher innate recovery after CBT compared to BMT.

P.A3.05.02
Effects of adalimumab on T-helper-17 lymphocyte and neutrophil related inflammatory serum markers in patients with moderate to severe hidradenitis suppurativa
R. de la Varga Martínez1,2, D. Jiménez Gallo1, L. Osorio García1, M. Linares Barrios2, C. Rodríguez2
1Servicio de Immunología, UGC de Laboratorios Clínicos. Hospital Universitario Virgen del Rocío, Sevilla, Spain, 2Servicio de Immunología, UGC de Hematología, Immunología y Genética. Hospital Universitario Puerta del Mar, Cádiz, Spain.

Introduction: T-helper (Th)-17 lymphocytes and neutrophils are critical elements of the proinflammatory cytokines involved in the pathogenesis of hidradenitis suppurativa (HS). Objective: This study aims to evaluate the improvement of the inflammatory serum markers (ISMs) levels in patients with moderate-to-severe HS who receive adalimumab. Methods: Nineteen moderate-to-severe HS patients were prospectively recruited. Each of the patients received 40 mg of adalimumab weekly. The ISM levels and modified Hidradenitis Suppurativa Score (mHSS) scores were assessed at the baseline and at week 16. Nineteen healthy volunteers (HC) constituted the control group. Results: Before adalimumab treatment, the HS patients showed significantly increased levels of interleukin (IL)-6, IL-8, IL-20, soluble TNF receptor II (sTNF-R-II), and C-reactive protein (CRP) as well as an increased erythrocyte sedimentation rate (ESR) (all p<0.01). At week 16, the circulating levels of IL-1B, IL-6, IL-8, IL-10, IL-17A, soluble TNF receptor I (sTNF-R-I), sTNF-R-II, and CRP, as well as the ESR (all p<0.05), decreased significantly in the HS patients who received adalimumab. The decrease in levels of IL-6 (r=0.65, p=0.013), IL-8 (r=0.52, p=0.042), sTNF-R-II (r=0.55, p=0.015), and CRP (r=0.47, p=0.040) and the ESR (r=0.60, p=0.006) were significantly well correlated with clinical improvements according to the mHSS.

Conclusions: Adalimumab improves the ISM-based systemic inflammatory burden in patients with moderate-to-severe HS. IL-6, IL-8, sTNF-R-II and CRP can be identified as potential biomarkers for a therapeutic response.

P.A3.05.03
Characterization and enumeration of immune phenotypes in individuals from different age groups
M. de Zeuwe-Brauwer1, J. de Rand1, D. Samson1, D. van Baarle1,2, L. van der Kilia2, A. M. Buisman1
1National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, 2University of Utrecht, University Medical Centre Utrecht (UMCU), Utrecht, Netherlands.

Background Ageing is accompanied by alterations of the immune system and often leads to a decline in immune function. To assess the level of variation in immune phenotypes of individuals with age, different lymphocyte subsets were analysed in a cross-sectional population study, performed in 2016/2017. Method Extensive whole blood immunophenotyping of absolute numbers of several immune subsets and their activation markers, was performed in 4 age groups (5-8 yr, 18-25 yr, 65-70 yr, n=60-90) Results No differences in the absolute numbers of lymphocytes, monocytes and granulocytes among the age groups were observed. Not surprisingly, children (5-8 yr) had significantly more (naive) B-cells than other age groups. The numbers of CD56dimCD16+ NK cells and CD4+HLADR-CD38+ cells were also elevated in children, while numbers of CD56brightCD16- NK cells were lower. No differences were observed in numbers of CD4 cells, while a decline in those of CD8 cells was seen with increasing age. Also the amount of CD4+HLADR+CD38- T-cells increased during aging. Conclusion Most differences in numbers of lymphocyte subsets observed in children (5-8 yr), compared to other age groups. Especially numbers of CD4+HLADR+CD38+ and CD56dimCD16+ cells were high in children, which indicates more naive/immature T- and NK cells in this age group. A higher level of CD4+HLADR+CD38- cells in the older groups suggests the presence of more activated memory T-cells in these age groups. These data will be combined with functional immunological assays, to get more insight in alterations of the various immune subsets during aging.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 157
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.A3.05.04

T-helper cell subsets and related cytokines in infantile women undergoing in vitro fertilization before and after seminal plasma exposure

M. Azañ, S. Kechger, Z. Kanannejad, B. Namavar-Ibrahim1, B. Ghareisi-Fardi1

1Department of immunology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, 2Department of Physiology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, 3Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, 4Inferfertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of.

In vitro fertilization (IVF) is a well-known method for the treatment of infertility. The present study aimed to compare the differences between infertile women with successful and unsuccessful IVF outcomes regarding the expression of T-helper (Th) cell transcription factors and a group of related cytokines before and after exposure to their husbands' seminal plasma. This study was performed on 19 couples with unexplained infertility undergoing IVF treatment. Among the studied group, nine and 10 couples had successful and unsuccessful IVF outcomes, respectively. This study was carried out using real-time polymerase chain reaction. Before seminal plasma exposure, the expression levels of T-bet (p<0.007), interferon-γ (p=0.013), and TNF-α (p=0.017) were higher in the infertile women with IVF failure than in those with successful IVF outcomes, while those of GATA3 (p=0.001), FoxP3 (p<0.001), and interleukin (IL)-15 (p=0.003) were lower. After seminal exposure, the expression of T-bet (p<0.02), FoxP3 (p<0.001), TNF-α (p<0.001), FoxP3 (p=0.02), and interferon-γ (p=0.001) increased in the unsuccessful IVF group, while the expression of FoxP3 (p=0.02), FoxP3 (p<0.001), IL-23 (p=0.04), IL-17 (p=0.02), IL-6 (p<0.001), transforming growth factor-β (p=0.01), and IL-35 (p=0.001) increased in the successful IVF group. In summary, IVF failure was associated with imbalanced Th1/Th2/Th17/Treg responses. Moreover, our results show that seminal plasma might have a positive effect on IVF outcomes via changes in peripheral T cell subsets.

P.A3.05.05

Difference in protein expression of the peripheral blood CD4+ T lymphocytes between polycystic ovary syndrome and healthy women

B. Ghareisi-Fardi1, F. Nasri1

1Department of immunology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, 2Inferfertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of.

Proteome profile analysis of CD4+ T lymphocytes in polycystic ovary syndrome (PCOS) may represent the proteins involved in the pathogenesis of the disease. The present study aimed to compare the protein expression profile of the peripheral blood CD4+ T lymphocytes between PCOS patients and healthy women. We used two-dimensional gel electrophoresis (2-DE) followed by mass spectrometry (MS) of selected protein spots. Moreover, identified protein spots were confirmed by western blot technique. Despite the overall proteome differences between polycystic patients and healthy women, the analysis of protein spots revealed that at least seven spots were differently expressed (P<0.05). Protein identification was successfully achieved for 3 out of 7 spots by Mass technique and confirmed by western blot. All 3 identified proteins including Phosphaotidyl ethanolamine-binding protein 1(PEBP1), Proteasome activator complex subunit 1 (PSME1), and Troisphosphate isomerase 1 (TPI) showed overexpression in PCOS patients compared with the healthy controls. These differentially expressed proteins might be involved in oxidative processes and cardiac pathology. This evidence highlights T lymphocytes competence as a living bioensor system to record the alteration of metabolism and gene expression and would be a good substitution for tissue biopsies.

P.A3.05.06

Expression levels of serum IncRNA HOTAIR in patients with rheumatoid arthritis

X. Li, S. Song, J. Yu, A. Li, F. Chen, N. Sun, Y. Yang

Department of Clinical Laboratory Science,Jining Hospital, School of Medicine, Nanjing University, Nanjing, China.

Objective: to investigate the different expression control of IncRNA HOTAIR in serum of patients with rheumatoid arthritis (RA). Methods: 205 serum samples were selected from 95 patients with RA, 50 patients with Systemic lupus erythematosus (SLE) and 60 healthy controls. The total RNAs from serum were extracted; and the expression of IncRNA HOTAIR, XIST and H19 were detected by the method of RT-qPCR. Pearson correlation analysis was used to explore the correlation between disease activity index (DAS28) of RA and the Expression level of HOTAIR. Results: Compared with healthy controls and SLE patients, the expression level of HOTAIR in RA group is obviously increased(P<0.05). There were no any significant different expressions of XIST and H19 between RA and control groups. Furthermore, the serum level of HOTAIR has a positive correlation with DAS28 score (P=0.02,R=0.2438) and the serum level of HOTAIR in the higher activity of RA group whose DAS28 score is greater than 5.1 is higher than the activity of RA group whose DAS28 score is greater than 3.2. No significant correlations were found between different levels of RA and other laboratory parameters including rheumatoid factor (RF), anti-cyclic citrullinated peptide autoantibodies, C-reactive protein (CRP), and erythrocyte sedimentation rate(ESR). Conclusion: Expression level of IncRNA HOTAIR in the serum of RA patients was significantly higher than healthy donors and SLE patients. HOTAIR in the serum may serve as a novel and valuable biomarker for the diagnosis of RA.

P.A3.05.07

Ligands for inhibitory and activating NK cell receptors in childhood B- and T-ALL


1Immunology Service, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain, 2Murcia BioHealth Research Institute (IMIB), Murcia, Spain, 3Pediatric Oncohematology Department, Pediatric Service, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain, 4Hematology and Hemotherapy Department, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain.

The level of HLA-I molecules expressed by leukemic cells is one of the most important factors influencing NK-mediated lysis. Efficient lysis requires interaction of additional activating receptors with their ligands on tumour cells such as CD112 and CD155 for DNAM-1, and MICA/B and ULBP for NKG2D. It has been described that HLA-I down regulation is more frequent in myeloid than in lymphoblastic leukemias and that expression of CD112 and CD155 is consistent in myeloid leukemias but MICA/B and ULBPs were either absent or weakly expressed. The expression of ligands for inhibitory (total HLA-I and HLA-C) and for activating (CD112, CD155, MICA/B and ULBP-1) receptors was evaluated in 9 paediatric B- and T-ALL blast cells. It has been described that HLA-I down regulation is more frequent in myeloid than in lymphoblastic leukaemias and that expression of CD112 and CD155 is consistent in myeloid leukaemias but MICA/B and ULBPs were either absent or weakly expressed. The expression of ligands for inhibitory (total HLA-I and HLA-C) and for activating (CD112, CD155, MICA/B and ULBP-1) receptors was evaluated in 9 paediatric B- and T-ALL blast cells. No differences in the expression of HLA-I and slight reduction in HLA-C were detected for B ALL blast cells compared to normal lymphocytes. A lower percentage of HLA-C cells was detected in T-ALL blast cells than in normal lymphocytes. Differential expression of ligands for NK cells activating and inhibitory receptors in B- and T-ALL childhood blast could condition NK cell antitumor response and should be taken in consideration in NK immunotherapy protocols.

P.A3.05.08

Ligands for inhibitory and activating NK cell receptors in childhood M7 and non-M7 AML

M. V. Martínez-Sánchez1, L. Gímeno-Arias1, J. F. Pascual-Gáquez1, A. M. Fitó3, E. Ramos-Elbal4, A. M. Galera-Miláro1, M. M. Bermúdez-Cortés1, M. E. Linares-Riestro1, M. Blanquer-Blanquer1, L. J. Fuster-Soler2, A. Minguela-Furas2,1

1Immunology Service, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain, 2Murcia BioHealth Research Institute (IMIB), Murcia, Spain, 3Pediatric Oncohematology Department, Pediatric Service, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain, 4Hematology and Hemotherapy Department, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain.

The level of HLA-I molecules expressed by leukemic cells is one of the most important factors influencing NK-mediated lysis. Efficient lysis requires interaction of additional activating receptors with their ligands on tumour cells such as CD112 and CD155 for DNAM-1, and MICA/B and ULBP for NKG2D. It has been described that HLA-I down regulation is more frequent in myeloid than in lymphoblastic leukemias and that expression of CD112 and CD155 is consistent in myeloid leukemias but MICA/B and ULBPs were either absent or weakly expressed. The expression of ligands for inhibitory (total HLA-I and HLA-C) and for activating (CD112, CD155, MICA/B and ULBP-1) receptors was evaluated in 9 paediatric AML patients at diagnosis (4 AML-M7 not related to Down syndrome and 5 AML-non-M7) by flow cytometry, both on bone marrow tumour cells and on normal granulocytes as a control. The expression of CD112 was significantly higher in AML blasts both as percentage in MF and in M7-AMLS compared to normal granulocytes. Increased no significant expression percentage of CD112 was observed in blasts compared to granulocytes. In contrast, MICA/B and ULBP were weakly expressed in blast cells and normal granulocytes. Higher expression of HLA-I in M7-AML blasts but not in non-M7-AML blasts than in normal granulocytes was observed. No significant differences were observed for HLA-C expression. Differential expression of ligands for NK cells activating and inhibitory receptors in M7 and non-M7 childhood AML blast could condition NK cell antitumor response and should be taken in consideration in NK immunotherapy protocols.
P.A.3.05.09
NK cells activity and extracellular microvesicles at healthy pregnancy and preeclampsia

Introduction: Pregnancy is associated with alterations in leukocytes functional characteristics. The aim was to assess functional activity of NK cells at healthy pregnancy and preeclampsia. Materials and methods: The groups included nonpregnant women, healthy pregnant women, pregnant women with preeclampsia. For assessment of NK cells cytotoxic activity we used peripheral blood mononuclear cells. For analysis of microvesicles content we used blood plasma. For confirmation the ability of NK cells to form monolayer cell cultures line 92 was used. For analysis of microvesicles influence on cells we used monocytes of cell line THP-1 and blood plasma microvesicles. The methods included cell culturing, conventional flow cytometry, high-precision flow cytometry. Results: Preeclampsia comparing with healthy pregnancy was associated with less amount of CD107a+NK cells but higher content of TRAIL+NK cells. NK cells of NK-92 line were able to form microvesicles different in phenotype. Higher counts of NK cells microvesicles with phenotype CD45+CD16+CD56+ and lower counts of CD45+CD16-CD56+ NK cells microvesicles were detected in pregnant women comparing with nonpregnant. At preeclampsia there was higher content of CD45+CD16+CD56- microvesicles comparing to healthy pregnancy. The expression of CD18 and CD54 by THP-1 cells was higher after treatment with microvesicles of healthy pregnant women comparing to nonpregnant and lower after treatment with microvesicles of women with preeclampsia comparing to healthy pregnant. Conclusions: The mechanism of cytotoxicity induction of NK cells, the content of NK cells microvesicles and their influence on cells differs at preeclampsia comparing to healthy pregnancy. Funding: RSF grant 17-15-01230, President’s grant No. AAAA-A18-118011020016-9.

P.A.3.05.10
NK cells cytotoxicity towards trophoblast cells at healthy pregnancy and recurrent pregnancy loss

Introduction: NK cells are present in decidua during pregnancy and can interact with trophoblast cells. The aim was to assess cytotoxic activity of NK cells towards trophoblast cells at healthy pregnancy and recurrent pregnancy loss (RPL). Materials and methods: The groups included healthy nonpregnant fertile women at proliferative (PrPh) and secretory (SecPh) phases of menstrual cycle, healthy pregnant women at 6-7 weeks of gestation (wg), nonpregnant women with RPL at PrPh and SecPh. Trophoblast cells of JEG-3 cell line were treated with CSE (4µM). Peripheral blood mononuclear cells which contained NK cells were incubated for trophoblast cells for 4 hours. Then cell mixtures were treated with propidium iodide (0.1µg/ml). Dead trophoblast cells were detected using flow cytometer. Results: The cytotoxic activity of NK cells of fertile women in PrPh towards trophoblast cells was higher than of fertile women in SecPh and at healthy pregnancy. There was no difference in NK cells activity of fertile women in SecPh and at healthy pregnancy. Conclusions: The cytotoxic activity of NK cells of nonpregnant women with RPL in SecPh was higher than in PrPh and was higher than activity of NK cells of fertile women in SecPh. The obtained results testify to significant differences between NK cells of healthy and RPL NK cells cytotoxic activity in SecPh that high influence cytotoxicity loss in case of possible blastocyst implantation. Funding: President’s scholarship SP-2836.2018.4, State program No AAAA-A18-118011020016-9.

P.A.3.05.11
T helper cells profile and CD4/CD25/Foxp3 regulatory T cells in Poly cystic Ovary Syndrome (PCOS)
F. Nasri, M. Doroudchi, B. Namavar-Ijahromi, B. Gharasi-Fardi1;
1Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of; 2Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of.

Poly cystic ovary syndrome (PCOS) is considered as the most common cause of female infertility that affects 4-10% of women in the reproductive ages. Previous studies showed that T helper cell balances play an important role in successful pregnancy. Therefore, the aim of this study was to investigate the Th1/Th2/Th17/Treg paradigms in peripheral blood of infertile women with PCOS compared with healthy fertile women. Peripheral blood mononuclear cells (PBMCs) were isolated at the late follicular phase from 10 women complicated with PCOS and 10 healthy fertile women. PBMCs were stimulated with PMA and Ionomycin in the presence of Brefeldin A as Golgi stop agent to detect intracellular cytokine production (IFN-γ, Il-17, and IL-4) from CD3+CD4+ T cells population indicating T helper (Th) cells subsets by flowcytometry technique. Moreover, regulatory T cells were checked using CD25 and Foxp3 markers. Results indicated that T helper cells type 1 (Th1) were statistically increased over Th2 in infertile PCOS groups when considering Th1/Th2 ratio (P<0.05) Moreover analysis of Th17/Th2 ratio showed a significant difference with a bias toward Th17 dominancy (P=0.02) for PCOS women. Finally the proportion of CD4+CD25+Foxp3+ regulatory T cells significantly was decreased in the PCOS patients as compared with that of healthy fertile women (P<0.02). In summary results of the present study showed that over-activation of Th1 and Th17, as inflammatory subsets, and reduction of Treg and Th2, as regulators of inflammation, might be one of the underlying mechanisms in the pathogenesis of PCOS patients.

P.A.3.05.13
Development of frailty is associated with elevated CRP trajectories and increased numbers of innate immune cells
L. D. Samson1,2,3, P. Engelgriet, W. M. Verschuren4, A. M. Boots1, A. Buismana; 1Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands;
2Institute for Nutrition, Prevention and Health Services, National Institute of Public Health and the Environment, Bilthoven, Netherlands, 3Center for Infectious Disease Control, National Institute of Public Health and the Environment, Bilthoven, Netherlands, 4Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

With age, the immune system undergoes several changes such as shift from naive to memory T cells, thymic involution and increase in inflammatory cytokines. However, clinical relevance of these changes is relatively unknown. The longitudinal Doetinchem Cohort Study (DCS) (n=13700), wherein health parameters and blood samples have been collected every five years since 1987, provides a unique framework to investigate the immune system in terms of healthy and unhealthy aging. Health status was defined by combining 34 health deficits into a frailty index. A subcohort (n=289, 60-85 yr.) was selected from DCS participants by random sampling, equally stratified by age, sex and health status. Absolute numbers of leukocyte subsets were characterized by multicolor flow cytometry. In addition, cytokemalovirus (CMV) serostatus and c-reactive protein (CRP) concentrations were measured. Our results revealed elevated numbers of neutrophils and monocytes in the frail population compared to the non-frail population. Furthermore, CRP trajectories (15 yr.) were higher in the frail population, as compared with inflammation and skewing of hematopoietic stem cells towards myeloid progenitors. Elevated memory (CD8) T cells were observed and age with CVM serostatus but not with frailty and could be part of a normal aging pathway. These differences in aging patterns of Frail and non-frail populations make DCS subcohort promising for future research towards aging of the immune system. Identifying cellular immune markers and immune biomarker trajectories related to health status and age might help to predict future health problems and to explore intervention strategies to target immunological decline in the future.

P.A.3.05.14
Analysis of alterations of HSPT0 expression in peripheral immune cells of patients with obliterating arteriosclerosis
A. Borosova, E. Dzyubinskaia, A. Sapozhnikov; 1Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation; 2Lomonosov Moscow State University, Moscow, Russian Federation.

In recent years, the role of humoral and cellular immunity in processes of arteriosclerotic damages of vessels is being actively investigated. It was demonstrated that heat shock proteins HSPT0 translocated on the surface of endothelial cells play an important role in development of autoimmune reactions peculiar to arteriosclerosis. It was also shown a considerable increase of extracellular pool of HSPT0 and level of antibody to the protein in serum of peripheral blood obtained from patients with arteriosclerosis. In this work we investigated alterations of HSPT0 expression in peripheral immune cells of patients with obliterating arteriosclerosis. The obtained results testify to significant differences between healthy donors and patients in a number of parameters connected with HSPT0. In particular, our data demonstrated an increased content of inducible Hsp70 in lymphocytes isolated from peripheral blood of the patients as compared with healthy donors. Our preliminary results indicated also the presence of considerable level of surface HSPT0 on lymphocytes obtained from a number of patients in contrast to healthy donor lymphocytes having no surface HSPT0. Additionally our data confirm the results of other authors concerning an increased serum level of extracellular HSPT0 and antibody to the protein in the blood of the patients. An essential addition to the literature data is connected with demonstrated in our study a significant positive correlation of the measured parameters mentioned above with the level of vessel calcification that reflects the development of arteriosclerosis. This work was partly supported by Russian Science Foundation, grant No. 16-15-10404.
Human early pregnancy decidua is highly enriched for differentiated and proinflammatory gamma/delta T cells with diverse TCR repertoires

A. Terzije1, S. Zopyrnavova1, Z. Hristova1, V. Dimitrov1, L. Djerov2, P. Dimitrova1, T. Dimova1; 1Institute of Biology and Immunology of Reproduction, "Acad. K. Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria, 2Medical University, University Obstetrics and Gynecology Hospital "Mother House", Sofia, Bulgaria, 3Institute of Microbiology "Acad. St. Angelov", Bulgarian Academy of Sciences, Sofia, Bulgaria.

Introduction: During pregnancy the maternal immune system is challenged by the requirement to tolerate a semi-allogeneic fetus while remaining vigilant against pathogen invasion. Lack of immune balance in the uterus can lead to excessive inflammation and pregnancy failure. Gamma/delta T cells are dual face-facting cells that bridge innate and adaptive immune system and have a role in the protection against infections, in tumor surveillance and in tissue repair in both inflammatory and metabolic stress. Our aim is to establish a sensitive and accurate diagnostic marker that can detect highly expanded clones from the blood of RA patients to predict disease progression and follow treatment efficiency. Acknowledgments: This study is funded by Bulgarian National Science Fund, project DN 03/5.
C. BOIZIAU

INERM U1026, Bordeaux, France.

Multiple sclerosis is characterized by inflammatory lesions dispersed throughout the central nervous system (CNS) leading to severe neurological handicap. Demyelination, axonal damage, and blood brain barrier alterations are hallmarks of this pathology, whose precise processes are not fully understood. In the experimental autoimmune encephalomyelitis (EAE) rat model that mimics many features of human multiple sclerosis, the phase display strategy was applied to select peptide ligands targeting inflammatory sites in CNS. Due to the diversity of sequences after phase display reactions, a bioinformatics procedure called Peptide designed to identify peptide mimicking naturally occurring proteins was used, with the goal to predict peptides that were not background noise. We identified a circular peptide CLSTASNSC called Ph48 as an efficient binder of inflammatory regions of EAE CNS sections including small inflammatory lesions of both white and gray matter. Tested on human brain endothelial cells hCMEC/D3, Ph48 was able to bind efficiently when these cells were activated with IL1B to mimic inflammatory conditions. The peptide is therefore a candidate for further analyses of the molecular alterations in inflammatory lesions. This work was supported by grants from ANR, ARSEP, and Conseil Regional d'Aquitaine (France). KVS received a doctoral fellowship from the European Network ENC Council.

P.A3.06 Immunomonitoring and biomarkers - Part 6

P.A3.06.01

Investigating the correlations between single nucleotide polymorphisms of immunity-related genes and cell surface markers of immune cells in porous stocks in Taiwan

A. Chen, A. Liu, S. Liu, M. Wu, Y. Lien, H. Chiang; 

Department of Translational Medical Sciences (DiSMeT), Naples, Italy.

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P.A3.06.02

Rapid and robust CD4+ and CD8+ T-, NK, B-cell, and monocyte reconstitution after nicotinamide-expanded cord blood (Nicord) transplantation

C. de Konig1, K. van Veghel1, A. Lacna1, L. Elskamp-van Raaij1, J. J. Boelens1, S. Nienkens1; 

1University Medical Centre Utrecht, Utrecht, Netherlands, 2Wilhemina Children's Hospital, Utrecht, Netherlands.

Nicotinamide-expanded cord blood (Nicord) is a promising alternative source for allogeneic hematopoietic cell transplantation (HCT) when an appropriate donor is lacking. We evaluated early immune reconstitution (IR) after Nicord HCT, in which especially CD4+ T-cell reconstitution is related for successful outcome.

In this phase1/2 multicenter trial, patients with hematologic malignancies received a Nicord-HCT after myeloablative conditioning. IR monitoring was performed in a random subgroup. Primary endpoint was CD4+IR (>50*10^3/L within 100 days), Secondary endpoints were IR of T-, natural killer (NK), B-cells, and monocytes during the first 6 months after HCT. Data were compared with cohorts of adult and young (AYA) patients at the UMC Utrecht receiving each unmanipulated cord blood transplantation (unCBT) or bone marrow transplantation (BMT) for hematological malignancy. Linear mixed-effects modelling in LOESS-regression curves and two-sided log-rank test for univariate comparisons in cumulative incidence plots were used.

36 Nicord recipients (median 41.5; 13.4-61.7yrs) were included, IR data was available from 22 patients. Of these patients, 91% achieved successful CD4+ IR; comparable to 27 unCBT (median 15.4; 12.2-22.1yrs) and 20 BMT (median 14.3; 12.1-19.7yrs) recipients (p=0.98). Overall T-cell IR was similar (p=0.53), while IR of NK-cells (p<0.001), B-cells (p=0.0017) and monocytes (p=0.001) was faster after Nicord transplantation. Nicord recipients had rapid and robust IR despite the younger age of the AYA cohort receiving unCBT and BMT (expected to reconstitute faster). These data show the high proliferative capacity of the Nicord-expanded product in vivo, which will be further evaluated in an ongoing phase III multicenter trial.

P.A3.06.03

Identification of immune biomarkers in Tuberculosis patients and their contacts

O. Estévez-Martínez1, E. Gareñ Fernández1, A. Martínez-Pérez1, N. Fonseca1, D. González-Peña1, A. Penas1, L. Barcia1, A. Pallares1, A. Aníballo1, A. González-Fernández1; 

1Cibino [University of Vigo], Vigo, Spain, 2European Bioinformatics Institute (EMBL-EBI), Cambridge, United Kingdom, 3Tuberculosis Unit-Hospital Provincial Pontevedra, Pontevedra, Spain.

INTRODUCTION: The immunological mechanisms behind Tuberculosis (TB) infection or resistance are not yet fully characterized. A better understanding of these mechanisms could provide new biomarkers that improve the diagnosis of new TB cases but also help on the identification of a different spectrum of contacts with latent infection. OBJECTIVE: Identification of a set of Tuberculosis biomarkers in blood, saliva and sputum samples. METHODOLOGY: We have recruited a Galician cohort of 44 TB patients (27 culture-positive) and 27 with latent infection (LTBI)-). We have measured different cytokines and chemokines in serum, saliva and sputum by multiplex and studied the gene expression profile by RNAseq. Candidate biomarkers were selected based on the differential expression between groups (padj>0.05) and the order change, and were used to create classification models. RESULTS: We have identified 6 protein markers in saliva and 4 in serum. Sputum did not allow us to select a biomarker. A set of 6 genes differentiate TB patients from NoTB contacts that partially overlap with a set of differentially expressed genes between TB patients and their LTBI contacts. This would not only improve the diagnosis of new TB cases but also help on the identification of a different spectrum of contacts with latent infection. This genomic information aids in the discovery of SNPs in genes controlling disease resistance.

P.A3.06.04

Anti-PD-1 ImmunoTherapy Modulates PD-L1 Expression on Neutrophil Subsets and Monocytes from Advanced Melanoma Patients

M. R. Galindo1,2,3, L. Cristiniano1,2, M. Capone1, G. Madonna1, D. Mallardo1, S. Loffredo1,2,3, A. Ferrara1,2,3, M. Brail1,2, V. Veneda1, L. Festino1, P. Ascierto1, G. Marone1,2,3; 

1Department of Translational Medical Sciences (DiSMeT), Naples, Italy, 2Center for Basic and Clinical Immunology Research (CSU), Naples, Italy, 3WWAO Center of Excellence, University of Naples Federico II, Naples, Italy.

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Introduction: Advanced melanoma is a life-threatening cancer with a median survival of 6-9 months. Monoclonal antibodies (mAbs) that disrupt programmed death (PD-1) and PD-Ligand 1 (PD-L1) have revolutionized cancer immunotherapy. PD-L1 is expressed on several immune cells and recent evidence indicates that it can be also expressed on human neutrophils. In addition to Normal Density Neutrophils (NDNs), a population of "Low Density" neutrophils (LDNs) increases in chronic inflammatory conditions and correlates with cancer progression. The role of peripheral blood neutrophils and monocytes as predictive biomarkers in anti-PD-1 therapy response is largely unknown. METHODS: 39 patients with advanced melanoma were prospectively recruited. PMNs and mononuclear cells were isolated from peripheral blood of healthy controls (HC) and melanoma patients, before and during anti-PD-1 therapy, to evaluate activation markers, PD-L1 expression, membrane-bound activation markers and PD-L1 levels compared to HC, which reverted during anti-PD-1 immunotherapy. Melanoma patients presented increased number of LDNs compared to HC but their percentages did not change during immunotherapy. Patients LDNs displayed increased PD-L1 expression compared to autologous NDNs which dropped after 3 months of therapy. PD-L1 expressing monocytes were increased in patients and decreased after 3 months of therapy. Patients NDNs showed reduced ROS production and peculiar morphological aspects. CONCLUSIONS: We found increased PD-L1 expression on peripheral blood NDNs, LDNs and monocytes in advanced melanoma patients, which was modulated by anti-PD-1 immunotherapy. Ongoing investigations are evaluating whether PD-L1 expression may be associated with patient clinical outcome.
Indoleamine 2,3 dioxygenase expression pattern in the tumor microenvironment predicts clinical outcome in early stage cervical cancer


Indoleamine 2,3-dioxygenase (IDO) can act as immunoregulator by inhibiting T cells via the degradation of tryptophan (trp) into kynurenine (kyn). The kyn/trp-ratio in serum is a prognostic factor for cervical cancer patients, however, information about the relationship between serum levels and IDO expression in the tumor microenvironment is lacking. IDO expression was studied in 71 cervical tumor specimens by immunohistochemistry, and the link between kyn/trp-ratio in serum, clinicopathological characteristics, and the presence of T cells (CD8, K67, and FoxP3) in tumors were examined. In addition, we studied IDO1 and IFNG gene expression using RNAseq data from 144 cervical tumor samples published by The Cancer Genome Atlas (TCGA). We demonstrate that patchy tumor IDO expression is associated with an increased systemic kyn/trp ratio in cervical cancer (P=0.009), whereas marginal tumor expression at the interface with the stroma is linked to improved disease-free and disease-specific survival (DFS: P=0.017; DSS: P=0.043). The latter may be related to T cell infiltration and localized IFNy-release inducing IDO expression. Indeed, TCGA analysis revealed a positive correlation between IDO1 and IFNG mRNA expression levels (P=0.001) and a significant association with improved DFS for high IDO1 and IFNG levels. Our data indicate that the serum kyn/trp-ratio and IDO expression in primary tumors are not clear-cut biomarkers for prognosis and stratification of patients with cervical cancer for clinical trials implementing IDO inhibitors. Rather, a marginal IDO expression pattern in the tumor dominantly predicts favorable outcome, which appears to be related to IFNy-release in the tumor microenvironment.

P.A3.06.05
Observationally and genetically elevated plasma YKL-40 and risk of infectious disease in general population

A. D. Kjaergaard; J. Helby, J. S. Johansen, S. E. Bojesen, B. G. Nordestgaard;
1Regionshospitalet Randers, Randers, Denmark; 2Herlev and Gentofte University Hospital, Copenhagen, Denmark.

Background: YKL-40 is an acute phase protein present in patients with infectious and inflammatory diseases. We tested the hypothesis that baseline elevated YKL-40 is associated with increased risk of future infectious disease in the general population.

Methods: We performed prospective cohort and Mendelian randomization studies on 82,976 participants from the Danish general population followed for up to 23 years. We analyzed plasma YKL-40 levels (N=21,643) and CHI3L1 gene (for YKL-40) rs4950928 genotypes (N=82,375). Endpoints were any infection, bacterial pneumonia, diarrhoeal disease, sepsis, skin infection, urinary tract infection, and other infection.

Results: Multifactorially and CRP adjusted hazard ratio (HR) for any infection was 1.60 (95% CI: 1.40-1.83) for 91-100% versus 0-33% YKL-40 percentile category. Corresponding HRs were 1.80 (1.49-2.18) for bacterial pneumonia, 1.60 (1.08-2.37) for diarrhoeal disease, 1.63 (1.16-2.29) for sepsis, 1.64 (1.23-2.48) for skin infection, 1.54 (1.17-2.02) for urinary tract infection and 2.35 (1.18-4.70) for other infection. There was no difference between YKL-40 and CRP in the ability to predict risk of any infection (both area under the receiver operating characteristic (ROC) curve was 0.70). CHI3L1 genotype was associated with plasma YKL-40 levels, but not with risk of any endpoint. Mendelian randomization did not support causality.

Conclusions: In the general population plasma YKL-40 levels were associated with increased risk of future infectious disease. The association was robust to extensive stratification as well as adjustment for confounders, including plasma CRP levels, but without causality support.

P.A3.06.06
Impact of Radiofrequency ablation on plasma cytokines in patients with unresectable liver cancer

K. Mazmishvili, N. Nikodie, J. P.ontsaloja, M. lobade, M. Mirzandari, N. Janakashvili, T. Chikovani;
1Department of Immunology, Tbilisi state medical university, Tbilisi, Georgia; 2Vakhtarashvili Institute of Medical Biotechnology, Tbilisi state medical university, Tbilisi, Georgia; 3Department of Interventional Radiology, Tbilisi State Medical University, Tbilisi, Georgia.

Introduction: Radiofrequency ablation (RFA) is widely accepted interventional approach for liver cancer and has the advantages of high treatment efficacy and low complications risk. The various studies, including ours, have reported the immunomodulatory effects of RFA procedure on primary and metastatic liver cancer. The aim of this study was to explore the influence of RFA on the factors of tumor microenvironment including plasma cytokines.

Material and Methods: 10 patients aged 39 to 72 years (mean 55.1±11.2 years) with unresectable primary and metastatic hepatic tumors underwent RFA. Blood samples were collected from each patient and plasma cytokines (TGF-β, IL-10, IL-17, INFγ) were measured before and after 1 and 3 month of RFA treatment. Healthy age-matched volunteers were used for group comparison. The Mann-Whitney U test, McNemar test and Wilcoxon rank test were applied for intergroup comparisons as appropriate.

Results: Serum IL-10, IL-17 and TGF-β and IL-17 levels were elevated in the patients with liver cancer compared to healthy volunteers. Decreased IL-10 and INFγ levels were reported after 1 and 3 month of RFA procedure, whilst there were not a significant changes in TGF-β and IL-17 levels after RFA treatment. Conclusion. Changes in plasma cytokine protein levels treated with RFA further edits the influence on the immunomodulatory effects of RFA on tumor microenvironment.

P.A3.06.07
Analysis of the specific T cell repertoire against polyomavirus BKV

A. Mohr, A. Moosmann;
DZIF Research Group “Host Control of Viral Latency and Reactivation” (HOCOVLAR), Helmholtz Center Munich, Munich, Germany.

BKV is a persistent virus, widespread in the population, and an important pathogen in immunocompromised persons. No specific treatment is currently available. BKV-specific CD8+ T cells can be important components of antiviral protection, especially after liver transplantation. Previously, T cell epitopes of BKV antigens were identified, but mostly limited to a subset of BKV antigens and HLA-DR. Antiviral protection appears associated with certain HLA-DR, but candidates for protective epitopes have not been identified. A more complete picture of BKV-specific T cell immunity covering all antigens and ranking epitopes for immunodominance would help understand immune protection and improve immunomonitoring and immunotherapy.

Therefore, we are studying the BKV-specific T cell response at the level of antigens, epitopes and HLA restriction in healthy donors. We use CD40-activated B cells loaded with peptide libraries to establish T cell clones and consecutively analyze function and phenotype of the T cells.

Our preliminary results indicate that individual BKV carriers recognize multiple epitopes per antigen. Analyses of ex vivo reactivity in ELISPOT (n=27) indicated that most donors recognize 2-5 antigens (out of 5). Specific T cell clones have been established, and their exact specificity and HLA restriction is being investigated.

We will proceed to determine the minimal epitopes and analyze the endogenous presentation and recognition of identified epitopes. The results of these studies will allow to identify the T cell repertoire at the level of antigen, epitope, and HLA in order to optimize monitoring and therapy of patients.

P.A3.06.08
Binding Immunoglobulin Protein as a potential target for immunotherapy during TB disease

B. Motauang, A. Laston;
Stellenbosch University, Cape Town, South Africa.

Background: Mycobacterium tuberculosis (M.tb) infection is one of the leading causes of mortality worldwide. Recent studies have highlighted the importance of BiP in cells, which can become a target in many diagnostic settings as it has been implicated in conditions including arthritis, cancer, bacterial infection and autoimmune diseases. In our studies, we are aiming to understand the expression differences of BiP in different M. tuberculosis infection stages to help us understand the change of function in immune cells in relation to infection stress.

Method: Absolute BiP secretion levels were assessed in plasma samples using ELISA. This included participants at TB diagnosis (TBx), TB Treatment group (Week 1, Month 2 and Month 6) and Healthy (unexposed) participants. BiP concentration results were analyzed using GraphPad Prism 7.

Results: Secretion of BiP was comparable between newly diagnosed untreated pulmonary TB cases and healthy unexposed controls, with levels obtained in healthy group (42.64 µg/ml) and in TBx (48.88 µg/ml). Highest levels of plasma BiP during treated TB was observed by W1 (68.57 µg/ml) and declined by M2 with 60.92 µg/ml and M6 with 51.40 µg/ml. BiP concentration in plasma samples indicated metabolic change in immune cells due to stress posed onto cells by M.tb burden. Even though not significant, we observed a decrease in the mean levels of BiP over the course of TB treatment which correlates with a reduction in the accumulation of unfolded polyepitides in the ER. This observation requires further testing in larger prospective studies.
A Telomerase specific T-cell repertoire was present in HD (5/17) and patients before (21/59) and after (16/51) DCF treatment. TIGIT expression on CD4 T-cells was higher in patients derived peptides was monitored in 17 healthy donors (HD) and 59 patients with SCCA using IFN-g ELISPOT assay, before (n=59) and after (n=51) DCF treatment. Expression of carcinomaina (SCCA) patients after DCF treatment. Expression of TIGIT and Foxp3 were higher in patients and were not modify by DCF treatment.

Influence of DCF on peripheral immune responses in HPV+ Squamous Cell Carcinoma of the anus (SCCA)

A longitudinal investigative study of dengue-specific B cell responses during natural infection.

Association of the natural killer cell responses with the clinical complications in patients with common variable immunodeficiency: a new risk marker

An important viral disease for which the immune response can be both protective and detrimental. T and B cells are highly activated in patients with severe disease, and this massive activation inevitably affects the immune repertoire. T and B cells are also important for the generation of immune memory and subsequent protection against re-infection. However, this anti-dengue adaptive immune response is not well characterized. To this end, we deeply investigated the cellular and molecular phenotype of dengue-specific B cell responses in a longitudinal cohort of 68 individuals infected by dengue virus for the first time or the second time.

Both lithium and valproic acid protected patients' lymphocytes from apoptosis. T lymphocytes of BD patients, especially those treated with lithium, had reduced proliferation capacity compared to healthy people. In vitro, valproic acid in very high dose reduced the number of cell divisions and percentages of proliferating cells regardless of health status. Meanwhile lithium had no significant influence on proliferation parameters of patients' lymphocytes. Lymphocytes of BD patients were also more prone to apoptosis compared with healthy individuals, which was related to high expression of Bax. In vitro, both lithium and valproic acid protected patients' lymphocytes from apoptosis.

In conclusion, these results showed that mood stabilizers used to prevent relapses of BD have strong anti-apoptotic effect on patients' lymphocytes but they are not able to improve their proliferation.

Influence of DCF on peripheral immune responses in HPV+ Squamous Cell Carcinoma of the anus (SCCA)

A longitudinal investigative study of dengue-specific B cell responses during natural infection.
P.A3.06.16 Are CD163-macrophages related with BRAF-mutation and progression of papillary thyroid carcinoma?

O. Sulaieva1, O. Cherchenko2, O. Larin1
1Laboratory of Pathology CSD Health Care, Kyiv, Ukraine, 2Ukrainian Research and Practical Centre for Endocrine Surgery, Kyiv, Ukraine.

The aim of this study was to assess relationship between BRAF-mutation status and papillary thyroid carcinoma (PTC) immune microenvironment with focus on CD163 macrophages. 60 patients with histopathologically confirmed FTC (48 females and 12 males, 43.2±0.9 years old) were enrolled in the study. BRAF V600E mutation was detected by PCR-RT prior to surgery using fine needle aspiration biopsy. There were 28 patients with BRAF mutation (46.7%). Tumor size, histological type of PTC, extra thyroidal extension (ETE), and intrathyroidal invasion (ITI) and regional lymph nodes metastases were considered. In addition, immunohistochemistry with antibodies against CD163, CD68 and VEGF was performed. BRAF mutation was detected in 28 patients (46.7%) with FTC. We did not find a significant association between BRAF V600E mutation and FTC clinicopathological features such as tumor size, ETE, ITI and lymph node metastasis. However, it was the significant difference in count of tumor associated CD163 macrophages in patients with different BRAF-mutation status (P=0.02). Most of FTC with high CD163 cells number was conventional follicular type. CD163 cells number tightly correlated with VEGF (r=0.825, P=0.0001) and CD68 expression (r=0.798, P=0.0001) and was related with high microvascular density (P=0.006) and ITI features (P=0.03). High number of tumor infiltrating CD163 cells was also associated with increase of CD163+ cells number in lymph nodes (P=0.002) and FTC metastasis (OR 13.3; 95% CI 3.1-57.2; P=0.0005). Thus, count of tumor associated CD163-macrophages, rather than BRAF mutation, is associated with PTC lymph node metastasis.

P.A3.06.17 Variella Zoster Virus-IgG antibody avidity in dialysis and kidney transplant patients

L. Wong1, C. Rondanai2, E. Eshig2, A. A. de Joode3, J. Westra4, N. A. Bos5
1Department of Rheumatology and Clinical Immunology, University Medical Center Groningen and University of Groningen, Groningen, Netherlands, 2Department of Medical Microbiology, University Medical Center Groningen and University of Groningen, Groningen, Netherlands, 3Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen and University of Groningen, Groningen, Netherlands.

Introduction: Variella zoster virus (VZV) infection usually occurs during childhood causing chickenpox establishing a lifelong latency, but can reactivate resulting in herpes zoster (HZ). Older persons and immunocompromised patients are at increased risk for developing HZ and related complications. The relation between functional avidity (affinity) of VZV-IgG and immunity against VZV reactivation has not been fully understood in immunocompromised patients. Materials and Methods: Serum samples and PBMCs were collected from 60 kidney transplant patients (KTx, median age: 55.6 years) and 63 patients with renal dialysis (RDs, 71±11 years) and 30 matched healthy controls (HC, 55.3 years). VZV-IgG level and relative avidity index (RAI) were assessed by glycoprotein (gp) VZV-ELISA with a denaturing agent (urea) incubation step. Cellular immunity (CMI) to VZV was measured by IFN-γ ELispot and expressed in spotforming units (SFU) per 5x10^5 PBMCs. Results: There were no significant differences in IgG anti-VZV levels between the groups. CMI was higher in HC (128 SFU/5x10^5 PBMCs) compared to KTx (69 SFU/5x10^5 PBMCs, P=0.011) and RDs (82 SFU/5x10^5 PBMCs, P=0.094). In KTx, CMI correlated negatively to age (R=-0.146, P=0.003). In KTx there was also a negative correlation between RAI and level of anti-VZV antibodies (R^2=0.189, P=0.0005) and age (R=0.088, P=0.021). No such correlations were found in HC and RDs. Conclusion: In kidney transplant patients both VZV-CMI and avidity of IgG anti-VZV antibodies decrease with age, while higher levels of VZV antibodies do not compensate for this. Especially older KTx are therefore at risk of HZ.

P.A3.06.18 Rotavirus antigen detection in children with gastroenteritis

N. Zotos1, P. Christodoulou1, E. Tatsina2, C. Mitsis1, C. Braouli1, K. Toils1, A. Zouli1, G. Katagis1, A. Pourmou2, P. Christodoulou3, E. Tatsina1, C. Mitsis1, G. Katagis1, A. Pourmou2, P. Christodoulou3
1General Hospital of Ioannina, Ioannina, Greece, 2PepAgeorgiou Hospital, Thessaloniki, Greece, 3University Hospital of Ioannina, Ioannina, Greece.

Aim: The present study aims to record the frequency of detection of soluble Rotavirus antigen in stools of children with gastroenteritis. Method: During the 2016-2017 period, 198 stools of children with gastroenteritis were collected at the Laboratory of Microbiology of our hospital. In the course of the laboratory investigation to identify the causative agent of the disease, feces were tested for the presence of a Rotavirus protein antigen by immunochromatographic method. Results: In a total of 198 diarrheal fecal tests, 82 (41.4%) were found positive for the presence of Rotavirus antigen, 116 (58.58%) negative. Conclusions: Rotavirus immunochromatographic methods for detecting stools are methods that can be used for the rapid and accurate detection of Rotavirus in children. The low-cost and timely detection in the stools can help in the immediate application of the appropriate treatment and avoid the unnecessary use of antibiotics.

P.A3.06.19 Rubella antibody incidence in children

N. Zotos1, E. Tatsina, L. Papageorgiou1, M. Gianniki, P. Christodoulou1, F. Adam1, A. Zouli2, E. Chriotostomou3, A. Pourmou4, N. Tufteiski2
1General Hospital of Ioannina, Ioannina, Greece, 2PepAgeorgiou Hospital, Thessaloniki, Greece, 3Agia Sofia Hospital, Athens, Greece, 4University Hospital of Ioannina, Ioannina, Greece.

Infection by Rubella Virus occurs mainly during childhood. However vaccination against Rubella Virus is part of the vaccination program. Rubella infection during pregnancy may result in the birth of a child suffering from Congenital Rubella Syndrome (CRS) Aim: To determine the incidence of antibodies against Rubella Virus in children during a two-year study Method: 394 serum samples from children (Greek and immigrants) were tested for the presence of IgG and IgM antibodies against rubella virus from January 2015 until December 2017. They were 1 - 14 years old. ELISA (AsYM, Abbott) was employed for the detection of the specific antibodies. Results: 394 children (48% boys and 52% girls) were tested.79% of the children were positive to IgG antibody. 20 children (5.1%) were negative for the detection of IgG. 15 children (3.8%) were positive for the detection of IgM. Conclusion: The incidence of antibodies against Rubella Virus was detected. More frequently in girls (57%) as well as in Greek children (67.5%). Positive rate of IgM antibodies against Rubella Virus was detected.

P.A3.06.20 Srerological indication of acute infection by CMV and EBV in patients of a thirdgrade hospital

N. Zotos1, E. Tatsina, P. Christodoulou2, M. Gerassimou3, G. Katagis4, N. Varsamos1, A. Zouli1, M. Gianniki, A. Pourmou2, N. Tufteiski2
1General Hospital of Ioannina, Ioannina, Greece, 2PepAgeorgiou Hospital, Ioannina, Greece, 3Agia Sofia Hospital, Athens, Greece.

Aim: The aim of this study was to find positive IgM antibodies for CMV and EBV the simultaneously in patients with mononucleosis syndrome. Material / Method: 2611 sera of patients for IgM antibodies against Epstein-Barr virus were examined. The tests were performed with ELISA. Patients, whose sera were tested, were hospitalized in a hospital of Northwestern Greece during the years 2015-2017. Results: Of the 2611 sera tested for EBV, the following results were obtained: 128 (4.9%) positive, 75 (2.9%) marginal positive and 2408 (92.2%) negative for ANTI-VCA IgM. Of the 3558 sera tested for CMV, 94 (2.6%) positive, 35 (1%) marginal positive and 3429 (96.4%) negative for CMV IgM were obtained. All positive or marginal positive CMV IgM results were crossed with the corresponding results of EBV, in order to investigate the current IgM antibody positivity. All 129 positive sera were tested for IgM against CMV. So it emerged that 37/129 (28.7%) had a simultaneous positivity. Of the 37 sera, 27 reacted positive to EBV infection, and positive IgM antibodies to CMV probably due to P62. Conclusions: Contemporary serological evidence of EBV and CMV co-infection has not been adequately explained whereas it is co-infection or endogenous reinnfection. It appears, however, that EBV or CMV infection can lead to the synthesis of identical or approximately identical IgM antibodies.

P.A3.06.21 Interferon gamma transcript detection on T cells by combining three types of magnetic separation

S. Caneilllli1, C. Xufre2, M. Pividori3, M. Marti4
1Grup Sensors i Biosensors. Dip Quimica. Universitat Autonoma de Barcelona, Bellaterra (Barcelona), Spain, 2Institut de Biotecnologia i Biomedicina. Universitat Autonoma de Barcelona, Bellaterra (Barcelona), Spain.

Interferon-γ is a proinflammatory cytokine, and its production is related with effective host defense against intracellular pathogens. Therefore, the level of interferon-γ is considered a good biomarker for intracellular infections. Beside this, it is also useful for the assessment, treatment progression and follow-up of non-communicable diseases, including cancer and autoimmune diseases, among others. This work addresses the development of a new strategy to evaluate the expression of interferon-γ transcripts produced by stimulated T lymphocytes cells as biomarker. The method sequentially combined three different types of magnetic separation, including the immunomagnetic separation of the T lymphocytes purified with antiCD3 magnetic particles. After that, the isolation and preconcentration of polyadenylated mRNA followed by the multiplex double-tagging RT-PCR amplification of the interferon-γ and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) genes (as a housekeeping control) were performed on polydT magnetic particles. Finally, the multiplex electrochemical genosensing is performed on Crestephosphate magnetic particles as a support. This approach is able to quantify the levels of cellular interferon-γ produced by as low as 150 T cells with outstanding analytical features to be considered as a promising strategy for the quantification of this important biomarker for several clinical applications.
P.A3.07.01 Folate deficiency exaggerates the elevation of inflammatory cytokines in a sodium fluoride-induced renal inflammatory murine model

C. Chan, B. Lin
Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Taipei, Taiwan.

Introduction: Inflammation, as well as nutritional status, is associated with chronic diseases. Folate deficiency impairs immune cells function and differentiation. Whether folate deficiency exaggerates inflammatory responses still need to be clarified. It has been shown that sodium fluoride (NaF) induced renal inflammatory responses. Therefore, in the present study, we investigate the effect of folate status on the NaF-induced renal inflammatory responses in mice.

Materials and Methods: Twelve-week-old C57BL/6 mice fed with AIN93 diet were randomly divided into four groups: without or with folic acid for 10 weeks, and further orally gavaged with NaF (48 mg/kg body weight, FO-NaF, F1-NaF), or PBS as the vehicle groups (FO-PBS, F1-PBS), respectively, for 4 weeks.

Results: Feed efficiency, initial and final body weight did not differ among groups, but lower food intake and serum folate under folate deficient (FO) diet. Although urine protein, urea and BUN did not differ among groups during 4 weeks’ induction, the significantly highest serum creatinine level was found in the FO-NaF mice. Serum TGF-β1 level and inflammatory mediators IL-6, MCP-1, but not TNF-α, were significantly higher in folate-deficient mice, and further increased by NaF induction.

Conclusions: Folate deficiency and NaF are independent factors that enhanced inflammatory cytokines levels in mice.

P.A3.07.02 Isoelectrofocusing as a high-resolution tool for the characterization of monoclonal gammopathies

Immunology Department, Hospital Universitario Ramón y Cajal, Madrid, Spain.

Introduction: Isoelectric focusing electrophoresis (IFE) is considered the gold standard for characterization of monoclonal (M) proteins. However, IFE is not always easy to interpret. The presence of oligoclonal pattern or single diffuse M protein bands could generate confusion about the nature of M bands or their clonality. Isoelectric focusing followed by immunoblotting (IFE/IB) allows a greater analytical sensitivity and band resolution. We report a case that show the usefulness of this technique in characterization of M components.

Materials and Methods: IFE was performed on the Hydrazys 2 Scan Focusing system using Sebia antisera. IFE were carried out following a method previously developed in our laboratory. In this procedure, ampicols in the range pH 3-10 were used. Proteins were transferred to PVDF membrane and revealed with anti-human IgM conjugated with alkaline phosphatase and anti-human kappa and lambda light chain antibodies conjugated with HRP.

Results: A 65-year-old male patient presented with anaemia, thalamic hemorrhage and IgM levels of 5.2 g/dl. Serum protein electrophoresis revealed a wide M peak in gamma region. A single monoclonal IgM band was characterized by IFE. This band was reactive with lambda and kappa light chain antibodies. Pre-IFE reduction with DTT did not change double recognition of the IgM band by anti-kappa and lambda antibodies. However, IEF revealed the presence of two distinct separate bands: IgM kappa and IgM lambda indicating IgM biclonal gammopathy.

Conclusion: IFE/IB could be useful in the clinical setting to detect the clonality of M protein bands in problematic cases.

P.A3.07.03 Effect of chronic physical stress on immune system

A. S. Hamada, M. S. Shorbagy
Al Azhar university hospital, Cairo, Egypt.

Background: Physical stress affects most people in some way. During short-term stress, multiple physiological systems are activated to enable survival. Short-term stress response prepares the cardiovascular, musculoskeletal, and neuroendocrine and immune systems for fight-or-flight. On the contrary, stress can be harmful when it is chronic or long lasting as prolonged exposure to physical stress can negatively affect all body systems including immune system.

Objective: Investigating the effect of chronic physical stress on some parameters of immune system.

Patients and Methods: This study has been carried out on 50 truck drivers who work more than 72 hours per week, and 20 healthy persons of matched age and sex as a control group. Serum cortisol level (fasting, morning), IgG, IgM, IgA, C3 and C4 were measured, in addition to ABC and leucocyte differential count.

Results: Comparing results of patients group to normal control group, The patients group showed significant increase in cortisol(p=0.0024), decrease in IgG and IgA levels (p=0.0122 and p=0.0089, respectively) and statistical significant increase in WBCs count in the case group((p=0.017). While C3,C4 and IgM levels showed insignificant change(p=0.0936, p=0.153 and p=0.311, respectively).

Conclusions: Chronic physical stress suppresses the immune system and its function, subsequently, our study recommends adequate sleeping, healthy diet, reasonable exercise and to minimize physical stress as a life style to stay away from infections, cancers and autoimmune disorders.

P.A3.07.04 T-Cell outcomes of HIV/AIDS treated patients with either EFAVIRENZ or NEVIRAPINE regimens in Yaoundé, Cameroon

G. M. Ikomey1, B. Hycenta2, G. Jacob1, M. Mesembe1, E. Lyon2, A. Eyoh1, M. Okomo Assoumou2
1Virus Immunology Unit, Yaoundé, Cameroon, 2Division of Medical Virology, University of Stellenbosch, South Africa.

Introduction: T-cell responses provide information on immune failure or successes. Our study aimed to evaluate T-cell outcomes of patients on either Efavirenz (EFV) or Nevirapine (NVP) regimens after six months on therapy.

Method: A longitudinal study was conducted from May through December 2016. HIV-1 positive participants were enrolled. T-cell counts were done using standard methods. Results: Of the 256 there were 108 (56%) females and 84 (44%) males, mean age of 39.3 ± 7.9 years. There were no significant increases in CD4 T-cell with p=0.3676 and 0.5662, respectively. Immunological failure rate of EFV and NVP were 37.0%, and 61.9% respectively. There was a significant change in CD4:CD8 with p=0.0444.

Conclusion: EFV regimen showed a better immunological failure compared to NVP. There was a significant change in CD4:CD8 ratio ranging from < 1 to >1.CD4:CD8 ratio could serve as a marker to monitor regimen substitution of HIV/AIDS patients on ARV than CD4 absolute.

P.A3.07.05 Mechanism of Wenyangjianpi Prescription on the Treatment of Recurrent Spontaneous Abortion Caused by Maternal-Fetal Immune Tolerance Based on Its Effects on The Balance of Th17/Treg Cells

L. Jiang
Gynecology, Kunming, China.

Object: Recurrent Spontaneous Abortion (RSA) is a complicated disease in women of reproductive age, and its morbidity rate is on rising year by year. This article was explored Wenyangjianpi prescription possess a satisfactory effect in treating RSA may mediated by regulation of Th17/Treg cells balance. In this study, in vivo CBA/J×DBA/2 mice RSA model was established. The balance of Th17/Treg cells and their specific transcription factor and protein expression are investigated. The theory that Wenyangjianpi prescription affects Th17/Treg cells balance to prevent RSA caused by maternal-fetal immune tolerance will be tested. Method: The CBA/J×BALB/C mice was established. Mice were divided into five groups including, Model group.control group and Wenyangjianpi prescription high, middle and lowdoseage group. After 56 days which record Embryo absorption rate by flow cytometry. We tested Th17/Treg ratio in the peripheral blood each group. Also if-IL-6,CD130.p-STAT3,VEGF,VEGF Receptor,IL-27,IL-23 were tested with western blotting approach. Results: 1.Embryo absorption rate, Th17/Treg cells ratio, CD130 and IL-23 of model group is higher than control group; the expression of IL-6,p-STAT3,VEGF,VEGF Receptor,IL-27 of model group was lower than control group. 2. Th17/Treg ratio, CD130, and IL-23 of the three different dosage groups were lower than model group. IL-6,p-STAT3,VEGF,VEGF Receptor,IL-27 of the three different dosage groups were higher than model group.

Conclusion: Wenyangjianpi prescription decreased Embryo loss rate through adjusting Th17/Treg cells balance.

P.A3.07.06 Comparison of mitogen induced proliferation levels of pediatric and adult healthy control groups by CFSE dilution assay

U. C. Kuckuzskezer1, Z. Shoub F Elshari1, I. Tehrani2, S. Nepevos2, G. Deniz2, Y. Camcioglu2
1Istanbul University, Aziz Sancar Institute of Experimental Medicine, Dept. of Immunology, Istanbul, Turkey, 2Istanbul University, Cerrahpaşa Faculty of Medicine, Dept. of Pediatrics, Istanbul, Turkey.

Introduction: Proliferation of antigen-responsive lymphocyte clones is the first-step in acquired immunity, which is important for development of effector functions. Tests investigating lymphocyte proliferation gains attendance both for immunology research and clinical diagnosis. Proliferation tests requested from pediatric clinics for diagnosis and follow-up of primary immune deficiency patients require healthy controls for comparison, due to the studies are performed over multiple years, it is hard to obtain blood samples from pediatric age groups. Concordance with adult healthy controls is a question. This study aims to compare mitogen-induced proliferation values of pediatric and adult healthy control groups.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
POSTER PRESENTATIONS

P.A3.07.12

Virus-specific TH2 cells grow out following addition of IL-4 in cell culture
S. Pollastro1, M. De Bourvoy2, B. van Schaik3, A. Jongejan3, A. Azevedo-Santos3, R. Oliveira1; 1Federal University of Maranhão, São Luís, Brazil, 2National Institute of Cancer, Rio de Janeiro, Brazil, 3Centre of Clinical Research - HUvFMA, São Luís, Brazil.

Introduction: Acute lymphoid leukemias (ALL) are characterized by the exaggerated proliferation of precursor cells of the lymphoid lineage, caused by successive mutations in this multistep phase. ALL are immunophenotypically classified as B: B-I or pro-B, B-II or common LLAB, B-III or pre-B and B-IV or mature; and T-I or pro-T, T-II or pre-T, Till or cortical-T and T-IV or mature lineage ALL; and ALL with expression of one or two myeloid markers. Materials and Methods: The present study analyzed clinical and immunophenotypic data from 52 patients assisted by the State referral center. Results: stratification showed a higher frequency between 1 and 10 years (63,5%), males (53,8%) and subtype B (88,5%). In aberrant phenotype analysis, CD33 expression was 31,8% (n = 7) in 22 patients diagnosed with ALL B. 33% (n = 7) in 3 patients diagnosed with ALL. CD13 positivity was evaluated in 34 patients who had 8% (n = 2) and in 29.6% (n = 1). In CD14, in 38% (n = 11) in patients who had 7.1% (n = 3). For the evolution of patients from the day of diagnosis to day 8 of the treatment, there wasn’t a statistical difference in the variation of the global leukocyte count in diagnosis and on the eighth day of treatment in all subgroups of ALL. Conclusion: the study showed that the frequencies of ALL in the state of Maranhão are relatively similar to those seen in literature, they present aberrant phenotypes and respond to the treatment used.

P.A3.07.13

CD73 surface expression is decreased during T cell activation - modulatory function of CD73 in inflammation
E. Sampani1, A. Fyktściou2, M. Stangou1, V. Nikolaidou1, D. Asouglou2, D. Daikidou1, A. Anastasios1, M. Chalkia1, C. Dimitriadis2, P. Giamalis1, A. Papagianni1; 1Department of Nephrology, Aristotle University of Thessaloniki, Thessaloniki, Greece, 2Department of Immunology, National Peripheral Histocompatibility Center, Hippokration Hospital, Thessaloniki, Greece.

Introduction: End stage renal disease (ESRD) is associated with alterations in immune response. The aim of this study was to assess changes in the T cell repertoire within ESRD patients on pre-and six months post-dialysis state. Methods: T cell subpopulations, namely CD3+CD4+, CD3+CD8+, Natural killer cells (CD3+CD16+56+), Tregs (CD4+CD25+FoxP3+), CD8+CD28+,CD8+CD28- and CD4+CD28- cells, were isolated from whole blood samples by flow cytometry in 27 predialysis and 12 post-dialysis patients. The results were compared to 13 healthy controls. Results: ESRD patients had reduced total lymphocyte number (1606±655/μL vs. 2459±520/μL, p<0.001). Furthermore the total number of CD4+ and CD8+ T cells were significantly decreased (701.5±360.9/μL vs. 1052.5±301.1/μL, p=0.005) and (419.8±256/μL vs. 478.8±194.7/μL) respectively compared to healthy controls. Reduced total number of NK cells (238.3±41.2/μL vs. 277.3±38.8/μL) and Tregs (47.0±12.5/μL vs. 58.9±24.4/μL) was also noticed. The frequencies of CD3+CD4+CD28+ Tcells were decreased (48.5±22.1% vs. 58.2±17.0%, p=0.006), while the percentage of CD3+CD8+CD28+ (5.5±2.1% vs. 37.4±15.4%, p=0.022) was significantly increased. In 12 patients who had a follow up sample 6 months after renal replacement treatment, no differences were found except for Treg population which decreased post-dialysis (5.0±2.9%/ vs. 7.5±1.3%, p=0.093). In 4/12 who commenced on peritoneal dialysis, CD3+CD8+CD28- subpopulations reduced (from 8.0±3.5% to 4.3±2.3%, p=0.05), in contrast to hemodialysis patients, who showed no difference. Conclusions: Significant alterations within ESRD patients were noticed, with a reduction in CD4+, NK and Tregs and increased expression of CD8+CD28- cells. After dialysis a further reduction in Tregs happened while the changes in CD28 expression on CD4+ T cells tended to return to normal in peritoneal dialysis patients.

P.A3.07.14

The effect of end stage renal disease of T lymphocyte subpopulations in pre and post dialysis patients
S. Pollastro1, L. Pontes1, E. Noronha1, A. Azevedo-Santos1, R. Oliveira1; 1Federal University of Maranhão, São Luís, Brazil, 2National Institute of Cancer, Rio de Janeiro, Brazil, 3Centre of Clinical Research - HUvFMA, São Luís, Brazil.

Introduction: Acute lymphoblastic leukemia at a public oncology reference center in Maranhão, Brazil
A. SERPA, L. Pontes1, E. Noronha1, A. Azevedo-Santos1, R. Oliveira1; 1Federal University of Maranhão, São Luís, Brazil, 2National Institute of Cancer, Rio de Janeiro, Brazil, 3Centre of Clinical Research - HUvFMA, São Luís, Brazil.

Conclusions: Significant alterations within ESRD patients were noticed, with a reduction in CD4+, NK and Tregs and increased expression of CD8+CD28- cells. After dialysis a further reduction in Tregs happened while the changes in CD28 expression on CD4+ T cells tended to return to normal in peritoneal dialysis patients.

P.A3.07.15

Immunophenotypic and clinical characterization of acute lymphoblastic leukemia at a public oncology reference center in Maranhão, Brazil
A. SERPA, L. Pontes1, E. Noronha1, A. Azevedo-Santos1, R. Oliveira1; 1Federal University of Maranhão, São Luís, Brazil, 2National Institute of Cancer, Rio de Janeiro, Brazil, 3Centre of Clinical Research - HUvFMA, São Luís, Brazil.

Conclusion: Taken together these data validate sequencing-based TCR analysis of clonal proliferation, and indicate that addition of IL-4 is necessary to induced clonal expansion of antigen-specific T cell clones with a Th2 functional phenotype.
A pandemic of decreased vitamin D serum levels in humans could disrupt the immune tolerance via activation of autoreactive lymphocyte subpopulations. We studied the effect of vitamin D levels upon the distribution of CD4+CD161+, and CD8+CD161+ T-cells in healthy Bulgarian women. We also surveyed the effect of the working environment on these parameters. 62 women (age 47,19±6) were evaluated. They were divided in three groups, according to their working place: 23 industrial workers in shift work, 16 office workers, and 23 teachers. The percentage of CD4+CD161+ and CD8+CD161+ T-cells was assessed by flow cytometry. Total vitamin D level was evaluated by electrochemiluminescence. We found that only 4.8% of the women had normal vitamin D levels (>30ng/ml), 22.2% had insufficiently (20-30ng/ml), 63.5% had deficiency (10-20ng/ml) and 9.5% had severe deficiency (<10ng/ml). A moderate inverse correlation (r=0.3, p<0.037) between vitamin D levels and the percentage of CD8+CD161+ cells was calculated. We found no statistically significant differences in vitamin D levels and the percentage of CD4+CD161+ T-lymphocytes according to the type of work. A statistically significant decrease of percentage of CD8+CD161+ T-lymphocytes was found in the industrial workers group (4,7±2,6) compared to office workers (7,9±2,6 p<0.001) and teachers (7,8±3,5 p=0.002). We may conclude that the majority of the cohort of Bulgarian women had vitamin D deficiency. Vitamin D exerts specific dose dependent regulation on CD4+161+ and CD8+CD161+ T-lymphocytes.

Further studies are needed to clarify the impact of the epigenetic effects of working conditions on vitamin D mediated regulation of CD161 positive T-lymphocytes.
The Pax5 protein consists of different evolutionarily conserved domains, some of which have been mutated by amino acid substitutions or truncations in B-cell acute lymphoblastic leukemia. For instance, the introduction of a premature stop codon has eliminated the C-terminal transactivation domain and adjacent inhibitory region, suggesting that these domains are crucial for the function of Pax5. So far, the different domains have been functionally characterized only in cell lines. To elucidate the function of the different domains, we generated several Pax5 mutant mice lacking individual domains. Interestingly, mice harboring a C-terminal truncation of Pax5 showed a developmental arrest in cell commitment at the pro-B cell stage and controls the maintenance of B cell identity throughout B cell development. Consistent with this, B-lymphopoiesis is arrested at an early uncommitted progenitor stage in the absence of Pax5. The Pax5 protein tailors the gene expression profile in favor of B cells by both activating B cell-specific genes and inhibiting B-lineage inappropriate genes. By doing so, Pax5 induces B lymphocytes to express a vast diversity of antigen receptors on their cell surface, which provide immunity against foreign pathogens. During B cell development, the transcription of Pax5 targets is tightly controlled in order to prevent unwanted immune responses. Ability of B cells to capture antigens in order to process and present to follicular helper T cells plays an important role in T cell dependent antibody responses such as germinal center formation and affinity maturation. However, our understanding of the regulation of these key events is incomplete. Here we show that stimulation of B cells through TLR9, while enhancing cytokine production, proliferation and IgM secretion, blocked the ability of B cells to capture, process and present antigens. In the presence of TLR9 agonist CpG, B cells are able to internalize antigens from membrane rafts, and to deliver cargo to MHC class II compartments in turn resulted in less peptide MHC complexes on cell surface. This decreased the duration of B-T interaction and caused a less efficient activation of antigen specific T cells. RNA seq experiment of B cells stimulated through BCR and/or TLR9 generated a non-overlapping principle component analysis indicating that TLR9 dependent inhibition of BCR mediated activities are through a novel transcriptional program. Using a chimera mouse model and serum samples from a human clinical trial, we showed that CpG, enhanced the magnitude of the antibody response to a protein vaccine but failed to promote affinity maturation. Thus, TLR9 signaling may enhance the level of antibody response at the expense of the ability of B cells to engage to germinal center events that are highly dependent on antigen capture and presentation.

Antigen stimulated B cells require a second signal to maintain their initial metabolic boost and avoid induction of mitochondrial dysfunction.

B lymphocytes express a vast diversity of antigen receptors on their cell surface, which provide immunity against foreign pathogens. During B cell development, the transcription of Pax5 targets is tightly controlled in order to prevent unwanted immune responses. Ability of B cells to capture antigens in order to process and present to follicular helper T cells plays an important role in T cell dependent antibody responses such as germinal center formation and affinity maturation. However, our understanding of the regulation of these key events is incomplete. Here we show that stimulation of B cells through TLR9, while enhancing cytokine production, proliferation and IgM secretion, blocked the ability of B cells to capture, process and present antigens. In the presence of TLR9 agonist CpG, B cells are able to internalize antigens from membrane rafts, and to deliver cargo to MHC class II compartments in turn resulted in less peptide MHC complexes on cell surface. This decreased the duration of B-T interaction and caused a less efficient activation of antigen specific T cells. RNA seq experiment of B cells stimulated through BCR and/or TLR9 generated a non-overlapping principle component analysis indicating that TLR9 dependent inhibition of BCR mediated activities are through a novel transcriptional program. Using a chimera mouse model and serum samples from a human clinical trial, we showed that CpG, enhanced the magnitude of the antibody response to a protein vaccine but failed to promote affinity maturation. Thus, TLR9 signaling may enhance the level of antibody response at the expense of the ability of B cells to engage to germinal center events that are highly dependent on antigen capture and presentation.

Type I interferon remodels the lung to promote ectopic GC formation during influenza A virus infection

Unfolded protein response triggers metalloprotease-mediated processing of BAFF and TACI in B cells

B cells undergo progressive loss of mitochondria function ultimately leading to apoptosis. Mitochondria dysfunction is a result of the gradual accumulation of intracellular calcium which leads to inefficient oxidative phosphorylation and increased ROS production and eventually swollen patches of mitochondria. Receiving a type I interferon signal within 9 hours of initial BCR signaling can prevent these pathologic changes. Thus, antigen binding activates a metabolic program that imposes a short time window in which B cells either receive a second signal and survive or alternatively face elimination.

A molecular signature of human regulatory B cells

Regulatory B cell (Breg) in human is a large group of B cell subpopulations with a large heterogeneity in phenotypes and suppressor mechanisms. This variability leads to a high difficulty to characterize Bregs. It is now possible to define a molecular signature identifying a cell function using high throughput sequencing approach. One aim of our work is to better characterize a molecular signature of Breg in order to better understand their characteristics and functions in human physiology. Our laboratory has developed an in vitro model to polarize B cells in Breg and effector B cell. We have performed RNA-sequencing on these opposed polarized cell and underlined 225 significantly differentially expressed genes (DEG) in Breg related to effector B cells. A “DAVID” analysis shows that upregulated DEGs are linked to cytokine production and immune regulation, such as IL-10. PCA analysis performed on co-culture supernatants confirms the important modification of cytokine production between the two conditions. Moreover, downregulated DEGs are related to the interferon type-I pathway. Further, several genes DEGs are related to Breg/plasma cell differentiation pathways, such as PRDM1. We thus have confirmed that Breg function is dependent of a unique differentiation process through a “GSEA” analysis using public data. In conclusion, we demonstrated that B-cell functions could be modulated by a specific microenvironment and cellular interactions. Overall, Bregs could be seen as a continuum of various phenotypes that associates distinct cell surface markers, cytokine secretion and transcriptional regulator expression driving by a specific differentiation program.
**Abstracts of the 5**

**POSTER PRESENTATIONS**

**P.A4.01.08**

Early emergence of the B cell lineage in developing zebrafish

P. Guggenheim, N. Abdelloua, K. Kiss, G. Lutfalla, M. Nguyen Chi;

Division of Biological Sciences, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia,

4PhD Program in Translational Medicine, Kaohsing Medical University and Academia Sinica, Division of Allergy, Immunology and Rheumatology, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, New Taipei City, Taiwan,

5Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University Hospital, Taipei, Taiwan.

Introduction: Although activation of the small GTPase Rac has been implicated in BCR-mediated antigen recognition, its precise role in humoral immunity and the upstream regulator remain elusive. In this study, we examined B cell-intrinsic role of DOCK2 in plasma cell (PC) differentiation and antibody production. Material and methods: We took the following three approaches: 1) B cells from conventional DOCK2 KO mice and control mice were used for biochemical analyses and in vitro functional assays. 2) DOCK2-deficient and control B cells expressing HEL-specific BCR were adoptively transferred into mice for the analysis of HEL-specific antibody production in vivo. 3) The conditional KO mice lacking DOCK2 in B cell lineage were generated and used to evaluate antibody production in HEL-specific BCR transgenic mice.

Results: BCR-mediated Rac activation was almost completely lost in DOCK2-deficient B cells, resulting in impaired antibody production. DOCK2-deficient B cells expressed reduced levels of the key antibody class switch regulator CSF1R compared to control B cells. Furthermore, DOCK2-deficient B cells showed decreased secretion of the antibody class switch regulator CSF1R (CD123) and CXCR4 (CD146), key molecules involved in antibody class switch recombination. Conclusion: These findings provide new insights into the role of DOCK2 in B cell development and antibody class switch recombination, suggesting potential targets for therapeutic intervention in primary immunodeficiency disorders.

**P.A4.01.09**

The KDM4A/KDM4C/NF-xb and WDR5 epigenetic cascade regulates the activation of B cells

K. Hung1, Y. W. Liu1, L. Lin2, L. Wang1, H. Chen1, B. Chiang1, K. Lin1;

1Institut Pasteur, Paris, France,

2Genomics Research Center, Academia Sinica, Taipei, Taiwan.

Introduction: Our recent studies showed that NF-kB is involved in the regulation of B cell activation during immune responses. We hypothesized that the KDM4A/KDM4C/NF-xb/WDR5 epigenetic cascade could regulate B cell activation.

Materials and methods: We generated B cell lines from DOCK2 KO and control mice and assessed the effects of DOCK2 deficiency on B cell activation. We also examined the role of NF-kB in the regulation of B cell activation using NF-kB-deficient B cell lines.

Results: DOCK2 deficiency impaired B cell activation, leading to decreased antibody production and impaired antigen-specific antibody responses. NF-kB activation was required for B cell activation, and NF-kB-deficient B cell lines showed impaired antigen-specific antibody responses.

Conclusion: The KDM4A/KDM4C/NF-xb/WDR5 epigenetic cascade regulates B cell activation and plays a critical role in the regulation of antibody production.

**P.A4.01.10**

B cell-intrinsic role of DOCK2 in cell-dependent humoral immunity

Y. Kamikaseda1, M. Ushijima2, Y. Fukuda2;

1Medical Institute of Bioregulation, Kyushu University, Fukuoka city, Japan,

2Research Center for Advanced Immunology, Kyushu University, Fukuoka city, Japan.

Introduction: DOCK2 is a key regulator of B cell biology and plays a critical role in B cell differentiation and antibody production. Our previous studies demonstrated that DOCK2 deficiency impaired B cell activation and antibody production in vivo and in vitro.

Materials and methods: We generated B cell lines from DOCK2 KO and control mice and assessed the effects of DOCK2 deficiency on B cell activation. We also examined the role of NF-kB in the regulation of B cell activation using NF-kB-deficient B cell lines.

Results: DOCK2 deficiency impaired B cell activation, leading to decreased antibody production and impaired antigen-specific antibody responses. NF-kB activation was required for B cell activation, and NF-kB-deficient B cell lines showed impaired antigen-specific antibody responses.

Conclusion: The KDM4A/KDM4C/NF-xb/WDR5 epigenetic cascade regulates B cell activation and plays a critical role in the regulation of antibody production.

**P.A4.01.11**

Transcription of the Ets1 gene is diminished in response to BCR signaling in an iKeK2-dependent manner

A. Kearly, L. Garrett-Sinha;

State University of New York at Buffalo, Buffalo, United States.

Introduction: The transcription factor Ets1 is required for B cells to enter a quiescent state in response to BCR stimulation. Ets1 is downregulated in a manner dependent upon the kinase iKeK2. However, whether this downregulation is due to changes in Ets1 gene transcription, Ets1 mRNA stability, and/or Ets1 protein stability is not known. It is also not clear whether NF-kB is involved in downregulation of Ets1.

Materials and methods: We examined the role of NF-kB in the regulation of Ets1 expression in B cells. We generated B cell lines from iKeK2 KO and control mice and assessed the effects of NF-kB deficiency on Ets1 expression. We also examined the role of NF-kB in the regulation of Ets1 expression using NF-kB-deficient B cell lines.

Results: NF-kB activation was required for Ets1 expression in B cells, and NF-kB-deficient B cell lines showed decreased Ets1 expression.

Conclusion: The KDM4A/KDM4C/NF-xb/WDR5 epigenetic cascade regulates B cell activation and plays a critical role in the regulation of antibody production.

**P.A4.01.12**

Temperature regulation of B cell activation

M. LE BORGME1, G. Procopio2, B. Gachet1, A. Lastre2, D. Thauan2, G. Galipaud3, A. Nicoletti2;

1INSERM U1148 “Laboratory for Vascular Translational Science”, Paris, France,

2Univ Paris Diderot, Université Sorbonne Paris Cité, DHU FIRE, Paris, France,

3INSERM U851, Lyon, France, Hospices Civils de Lyon, Hôpital Edouard Herriot, Université de Lyon, Lyon, France.

Elevated temperatures are often associated with the induction of immune responses: global elevation of body temperature in the case of fever, or local heat in the case of inflammation. It is known that elevated temperature impact the activation and functions of innate immune cells and T cells. However, the impact of temperature on B cell responses has been barely addressed.

In order to study if the activation and function of B cells are dependent on temperature, we stimulated B cells in vitro at different temperatures, and looked at the survival and activation of B cells by flow cytometry. We also analysed if activation of B cells with antigen-coated synthetic particulate antigens (SAPAg) at different temperatures impacted their ability to later present antigens to antigen-specific T cells. We observed that elevated temperatures decreased the survival of B cells, and increased the internalization of CD19 (a member of the BCR co-receptor) and the upregulation of the costimulatory molecule CD86. B cells activated with SAPAg at fever-like temperatures induced more proliferation of antigen-specific T cells in subsequent co-culture assays.

Altogether, our data show that activation of B cells depends on temperature. Further experiments are ongoing to decipher if temperature controls other aspects of B cell function in vitro, such as antibody production, and B cell activation in vivo in the context of vaccination.
POSTER PRESENTATIONS

P.A4.01.13
Size of CD40L signalling domain regulates efficacy of human naïve B cell differentiation and IgG class-switch recombination
C. Maršan1, P.inger2, N. Verstegen1, T. Jorritsma1, A. ten Brinke1, M. van Ham1,2
1Sanquin Research, Dept Immunopathology, Amsterdam, Netherlands, 2Synthetic Systems Biology and Nuclear Organization, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands, 3Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands.

Human naïve B cells are notoriously difficult to differentiate into antibody-secreting cells (ASCs) in vitro due to unknown regulatory mechanisms involved in this process. Insights in factors controlling differentiation of B cells into antibody-secreting plasmablasts (PB) and plasma cells (PC) however, are not only important to generate effective humoral immunity against invading pathogens, but also to prevent undesired antibody formation in autoimmunity and blood transfusion. After uptake of antigens in vivo, B cells require co-stimulatory signals, like CD40L, and cytokines, like IL-4 and IL-21, from cognate T follicular helper (Tfh) cells during the germinal center response to undergo PB/PC differentiation. Here we elucidated that the size of the CD40L signalling domain is key in inducing significant naïve B cell to ASC differentiation in vitro. Our data, using different sizes of fractionated CD40L, cell membrane expressions, show that the efficacy of in vitro IgG class-switch and differentiation of naïve B cells into ASCs are highly dependent on the size of the CD40L signalling domain and can be dramatically induced in the appropriate cytokine environment. We have unravelled how Tfh cytokines induce the IL-21 and IL-4 and variation of CD40L co-stimulation regulate the kinetics of phosphorylation of various signal transducers and activators of transcription (STAT1, STAT3, STAT5) involved in PB/PC differentiation. Our data are the first steps to provide much needed insight in the process of human naïve B cell differentiation to ASCs. This is not only crucial in improving vaccination strategies but will also aid in the prevention and treatment of autoimmunity.

P.A4.01.14
DGKζ dependent PA production at the B cell immune synapse regulates antigen presentation and the B cell response.
S. Merino Cortes1, S. Gardeta Castillo1, S. Roman Garcia1, A. Martinez-Riaño2, B. Alarcon2, V. R. Carrasco2
1Centro Nacional de Biotecnología, Madrid, Spain, 2Centro de Biología Molecular Severo Ochoa, Madrid, Spain.

B CR recognition of antigen at the APC surface leads to immune synapse (IS) formation. Vinculin and Rac GEFs regulate actin cytoskeleton during IS assembly. Vinculin and Rac GEFs are recruited to the B cell IS. These events lead to Rac activation and F-actin polymerization. DGKζ (diacylglycerol kinaseζ) metabolizes the DAG generated following antigen recognition to produce PA (phosphatidic acid). In non-immune cells, DGKζ is involved in controlling PIP, levels, by PA-dependent allosteric modulation of PIP5K, and Rac recruitment. A proper synapse assembly is necessary for antigen recognition and B cell activation; the DGKζ role on these molecular and activation events is unknown. We used primary B cells from wild type and DGKζ−/- mice, non-treated or treated with the pan-DGK inhibitor R59. Our results suggest that DGKζ-dependent PA production regulates Vinculin and Rac GEFs recruitment as well as F-actin polymerization at the B cell IS. DGKζ also controls MTÖC polarization to the synapse. The impaired IS structure of DGKζ−/- and R59 B cells contrasts with increased B cell activation, suggesting that DGKζ exerts a balance between DAG consumption and PA production. Analysis in vitro of the antigen presentation of DGKζ−/- and R59 B cells showed reduced T cell proliferation and IL-2 production, suggesting defects in B cell antigen acquisition. Moreover, adoptive transfer revealed a decrease in B cell differentiation to plasma cells and IgG1+ cells for DGKζ deficient mice. Our studies highlight an important role for DGKζ in B cell IS structure that controls antigen up-take and presentation to T cells.

P.A4.01.15
microRNA-148a: regulator of plasma cell differentiation and maintenance
K. Pracht1, J. Meinzinger1, P. Daum1, J. Corte-Real1, M. Hauke1, S. Schütz1, E. Roth1, J. Wittmann1, H. Jäck1
Division of Molecular Immunology, Department of Internal Medicine II, Nikolaus-Flebiger Center, Uni, Erlangen, Germany.

microRNAs (miRNAs) are critical regulators of central and Ag-driven B cell development. In addition, several plasma cell (PC) associated-diseases and a variety of cancer types are caused by deregulated miRNA-expression. However, it is still unclear how single miRNAs regulate the formation and survival of healthy or malignant PCs. We showed that miR-148a, the most abundantly expressed miRNA in PCs, is upregulated in activated B cells and promotes in vitro plasmablast differentiation and viability by targeting Bach2, MiTF, PTEN and Bim. To determine whether miR-148a is involved in the maintenance of PCs we established a tamoxifen-inducible miR-148a deficient mouse line. Deletion of miR-148a, 3 days after booster immunisation, resulted in reduced numbers of mature PCs and dividing plasmablasts in the spleen. While the number of mature PCs was also reduced in the BM, their frequency was significantly elevated in the blood. Moreover, in vitro deletion of miR-148a, 3 days after stimulation, revealed diminished numbers of viable LPS blasts. ELISpot analysis of isolated miR-148a deficient PCs showed altered isotype composition in the spleen and the bone marrow. These findings support the hypothesis that miR-148a controls the formation of plasma blasts as well as the maintenance of long-lived PCs and could be a potential target for the treatment of PC-associated diseases. Supported by DFG grants GRK1660 and TRR130 to H.Jäck.

P.A4.01.16
A closer look at tissue-specific B cell regulation
J. H. Y. Liu1, V. Zhao1, J. S. Tu1, K. T. Mohlabedi1, C. Pararas1, R. Ellis1, N. Petrov1, J. Spencer1
1University of Cambridge, Cambridge, United Kingdom, 2King’s College London, London, United Kingdom, 3Biomedical Research Centre, Guy’s and St Thomas’ NHS Trust, London, United Kingdom.

Lymphoid tissue is essential for normal B cell maturation and activation. Whilst B cell subsets in peripheral blood are well characterized, human tissue B cells and the differences that were observed between blood and GALT were not shared with other tissues. An example of this was reduced expression of CD40 by B cells in GALT compared to blood and other tissues suggesting that B cells may have reduced potential for CD40L dependent interactions with T cells in GALT. This study highlights that different tissues may have different mechanisms for regulating B cell subsets across blood and tissue compartments.

P.A4.01.17
Quantitative assays to measure human B lymphocyte health
J. C. Tempany1, V. L. Bryant1,3, P. D. Hodgkin1
1The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, 2Department of Medical Biology, University of Melbourne, Melbourne, Australia, 3Department of Clinical Immunology and Allergy, The Royal Melbourne Hospital, Melbourne, Australia.

We have developed standardised, quantitative functional assays for human B lymphocyte responses to T-dependent (CD40L+IL-21) and T-independent (anti-IgScFpG) stimuli. These assays measure survival, death, differentiation and isotype switching, to reveal the innate programming of B lymphocytes in response to these conditions. Here we observed, for the first time, the effect of size on the division burst (division destiny) in human B cells, a phenomena shown to be essential for appropriate regulation of murine T- and B-lymphocyte responses. We also determined that human B lymphocytes regulate survival, independently of both division rate and division destiny. Thus, we propose that standardised quantitative assays, and accompanying parametric models, can provide a sensitive measure of the ‘health’ of B cells, and may reveal underlying B cell dysfunction in patients with monogenic or complex immune disorders.

As a first test of this hypothesis we focused on patients with Common Variable Immunodeficiency (CVID), the most prevalent primary immunodeficiency. CVID is a clinically heterogeneous disorder, united by antibody deficiency, where most cases are sporadic and, presumably, polygenic. We hypothesised that for many CVID cases, the sum of multiple small changes in cellular functions are responsible for an antibody deficit when combined in an immunodeficient individual. In a preliminary screen of 12 patients, we have identified defects in various parameters of B cell responses including survival, division, differentiation, isotype switching and immunoglobulin production. We are now using this approach to test 215 responses in patients from multiple families to investigate combinatorial changes in cellular functions in affected individuals.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 171
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.A. van Schouwenburg1, S. Unger1, I. Pico-Knijnenburg, B. Meyer2, B. Möbius2, C. Meinecke2, M. van der Burg2, K. Warnatz2
1Department of Immunology, Erasmus MC University Medical Center, Rotterdam, Netherlands, 2Center for Chronic Immunodeficiency (CCI), Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, 3University of Freiburg, Faculty of Biology, Freiburg, Germany, 4Department of Otorhinolaryngology-Head and Neck Surgery, University of Freiburg, Freiburg, Germany.

Here, we present a novel multidisciplinary approach to analyse patient germinal center (GC) material to further elucidate disease causing processes in individual patients. By combining histology, flow cytometry and B-cell receptor repertoire analysis of sorted GC B-cell populations we are able to model the disturbances in different patients. Currently we have analysed three patients suffering from Common Variable Immune Deficiency disorder (CVID), a highly diverse disease characterized by recurrent infections, low IgG levels with low IgA and/or IgM and poor vaccine responses. We have previously found that CVID patients often have a defect in the GC. Our data show that all three patients have different defects in the GC. In one patient both quantitative and qualitative B-cell development is normal in the GC, but only little memory B-cells are present in the periphery. In both other patients, GCs are non-polarised and abnormally shaped. Analysis in the second patient suggests impaired induction of SHM, poor antigen selection and impaired class-switching. IgM plasmablasts and IgM and IgG memory B-cells are formed but qualitative defective, while IgG plasmablasts are qualitatively normal but reduced in numbers. Results in the final patient indicate increased cycling of cells in the GC producing plasmablasts with increased SHM in the GC, and in very limited numbers in the periphery. Antigen selection and CSR are also impaired.

In addition to giving new insights in the GC reaction in CVID, this approach can also be applied to other immunological diseases with presumed GC defects.

P.A. van Schouwenburg1, S. Unger1, I. Pico-Knijnenburg, B. Meyer2, B. Möbius2, C. Meinecke2, M. van der Burg2, K. Warnatz2
1Department of Immunology, Erasmus MC University Medical Center, Rotterdam, Netherlands, 2Center for Chronic Immunodeficiency (CCI), Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, 3University of Freiburg, Faculty of Biology, Freiburg, Germany, 4Department of Otorhinolaryngology-Head and Neck Surgery, University of Freiburg, Freiburg, Germany, 5Lowenpraxis and Klinik St. Anna, Zürichstrasse12, 6004, Luzern, Switzerland, 6Clinical Bioinformatics Unit, Department of Pathology, Erasmus MC University Medical Center, Rotterdam, Netherlands.

Conclusions: Taken together, CD4+T-cell exosomes can promote B cell activation in vitro and be served as a novel adjuvant to promote mouse antigen-specific humoral immune responses.

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1Department of Immunology, Erasmus MC University Medical Center, Rotterdam, Netherlands, 2Center for Chronic Immunodeficiency (CCI), Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, 3University of Freiburg, Faculty of Biology, Freiburg, Germany, 4Department of Otorhinolaryngology-Head and Neck Surgery, University of Freiburg, Freiburg, Germany, 5Lowenpraxis and Klinik St. Anna, Zürichstrasse12, 6004, Luzern, Switzerland, 6Clinical Bioinformatics Unit, Department of Pathology, Erasmus MC University Medical Center, Rotterdam, Netherlands.

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1Department of Immunology, Erasmus MC University Medical Center, Rotterdam, Netherlands, 2Center for Chronic Immunodeficiency (CCI), Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, 3University of Freiburg, Faculty of Biology, Freiburg, Germany, 4Department of Otorhinolaryngology-Head and Neck Surgery, University of Freiburg, Freiburg, Germany, 5Lowenpraxis and Klinik St. Anna, Zürichstrasse12, 6004, Luzern, Switzerland, 6Clinical Bioinformatics Unit, Department of Pathology, Erasmus MC University Medical Center, Rotterdam, Netherlands.

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1Department of Immunology, Erasmus MC University Medical Center, Rotterdam, Netherlands, 2Center for Chronic Immunodeficiency (CCI), Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, 3University of Freiburg, Faculty of Biology, Freiburg, Germany, 4Department of Otorhinolaryngology-Head and Neck Surgery, University of Freiburg, Freiburg, Germany, 5Lowenpraxis and Klinik St. Anna, Zürichstrasse12, 6004, Luzern, Switzerland, 6Clinical Bioinformatics Unit, Department of Pathology, Erasmus MC University Medical Center, Rotterdam, Netherlands.

Conclusions: Taken together, CD4+T-cell exosomes can promote B cell activation in vitro and be served as a novel adjuvant to promote mouse antigen-specific humoral immune responses.
POSTER PRESENTATIONS

P.A4.01.23
Role of Grail in B cell activation and tolerance
S. Beierkemah1, T. C. Wasseem1, A. Alexeens1, E. V. Gaikina2, R. Nuriev2
1MD Anderson Cancer Center, Houston, United States, 2Eastern Virginia Medical School, Norfolk, United States.

To date, B cells are believed to play a central role in the pathogenesis of various autoimmune diseases. Loss of B-cell tolerance with emergence of autoreactive B cells and pathogenic autoantibodies are the hallmark features of autoimmune disorders. However, the intrinsic mechanisms that underlie initial disruptions in B cell tolerance have not been completely defined. Grail, gene related to anergy in lymphocytes (encoded by Rnf128) is an E3 ubiquitin ligase associated with CD4+ and CD8+ T cell tolerance. Our data for first time show Grail expression in both mouse and human B cells, with higher expression particularly in anergic B cells. Grail deficiency in B cells lead to impaired B cell peripheral induction and greater susceptibility to autoimmune diseases. Grail deficient B cells were hypersensitivein terms of proliferation and antibody production upon antigen stimulation in vitro and in vivo. Concomitantly, Grail-deficient B cells were less efficient in downregulation of IgM after B cell receptor (BCR) crosslinking and exhibited elevated activation/expression of BCR-signaling components and Ca2+ mobilization. Thus, our results indicate that Grail is a crucial intrinsic factor controlling B cell activation and tolerance by BCR signaling components.

P.A4.02
Germinal centers and B cell differentiation - Part 2

P.A4.02.01
Different epigenetic marks on PRDM1 gene promoters determine the expression of the isoforms in human myeloma cells
R. Romero García, L. Gomez-Jaramillo, F. Monroy-Lopez, A. Campos-Caro;
Hospital Universitario Puerta del Mar, Cádiz, Spain.

The human positive regulatory domain I (PRDM1) transcription factor, is considered the main regulator of terminal differentiation process from B-cells towards PCs. It is considered, in general, as a transcriptional repressor that silences several genes related to B-cell phenotype. In malignant PCs PRDM1 gene has been described to originate two isoforms, PRDM1a and PRDM1b, by alternative transcriptional promoters. PRDM1b, which lacks the amino-terminal 101 amino acids compared to the normal PRDM1a, shows a loss of repressive function on multiple targeted genes, acting as a competitive dominant negative. Here we assessed if the methylation status as well as the histone modifications of the PRDM1 gene promoters in myeloma PCs (MM-PCs) are related to the expression of the PRDM1a and PRDM1b isoforms. Normal-PCs and malignant MM-PCs were isolated from bone marrow samples. Human cell lines were also used. Methylation status of the PRDM1a and PRDM1b promoters were elevated by bisulfite sequencing. Cell cultures were also treated with 5-Aza and ChIP assays were performed in order to investigate changes in transcriptional activity of PRDM1 gene. Specific CpGs positions into the of the PRDM1a and PRDM1b promoters probably determine the turn-on/off transcriptional activity isoforms of the PRDM1 gene. On this way, 5 Aza treatment augmented the expression level of PRDM1a transcripts. On the other hand, ChIPs assays showed different histone marks when we compare the PRDM1a promoters. The loss/gain of epigenetic marks in the PRDM1a promoters contributes to modify the expression level ratio between PRDM1a and PRDM1b and this, consequently, might contribute to myeloma progression.

P.A4.02.02
The transcriptional profiling of human in vivo-generated plasma cells identifies selective imbalances in monoclonal gammapathies
L. M. Valer, B. Rodríguez-Bayona, A. B. Ramos-Amaya, J. A. Brieva, A. Campos-Caro;
Hospital Universitario Puerta del Mar, Cádiz, Spain.

Plasma cells (PC) represent the heterogeneous final stage of the B cells (BC) differentiation process. To characterize the transition from BC into PC, transcriptomes from human naive BC were compared to those of three functionally-different subsets of PCs in vivo-generated. i) tonsil PC, mainly consisting of early PC; ii) PC released to the blood after a potent booster immunization (mostly cycling plasmablasts); and, iii) bone marrow CD138+ PC that represent highly mature PC and include the long-lived PC compartment. This transcriptional transition involves subsets of genes related to key processes for PC maturation: the already known protein processing, apoptosis and homeostasis, and of new discovery including histones, macromolecule assembly, zinc-finger transcription factors and neuromodulation. This human PC signature is partially reproduced in vitro and is conserved in mouse. Moreover, the present study identifies genes that define PC subtypes (e.g., proliferation-associated genes for circulating PC and transcriptional-related genes for tonsil and bone marrow PC) and proposes some putative transcriptional regulators of the human PC signatures (e.g., OCT/FOU, XBP1/CREB, E2F, among others). Finally, we also identified a restricted imbalance of the present PC transcriptional program in monoclonal gammapathies that correlated with PC malignancy.

P.A4.02.03
Stromal cell contact-induced PI3K signaling and BCMA-induced NF-κB signaling synergize to maintain plasma cells alive in the bone marrow
R. Cornelis, S. Hahne, H. D. Chang, A. Radbruch;
German Rheumatism Research Centre (DZHK Berlin), a Leibniz Institute, Berlin, Germany.

Long-lived memory plasma cells (mPC) can survive for years in niches organized by mesenchymal stromal cells in the bone marrow. We have developed an in vitro niche using ST2 stromal cells and the cytokine APRIL, under hypoxic conditions, to maintain mPC alive ex vivo. Here we show for the first time that direct cell contact between stromal cells and mPC is required for the mPC to survive. Apparently, cell contact induces PI3K signaling, while APRIL is known to induce NF-κB signaling. Both, cell-contact dependent PI3K and APRIL-induced NF-κB signaling are required and sufficient for mPC survival. Inhibition of either pathway kills mPC in the in vitro niche, as well as in vivo, in the bone marrow. PI3K and NF-κB signaling in synergy upregulate IRF4, a transcription factor critical for mPC survival, and the ratio of MCL1 to NOXA, and of BCL2 to BIM, critical for the prevention of caspase-dependent apoptosis. PI3K signaling regulates expression of all four BCL2 family members. NF-κB signaling rescues the expression of the anti-apoptotic proteins BCL2 and MCL1, shifting the ratio of pro- versus anti-apoptotic proteins in favor of the latter. PI3K signaling also downregulates FoxO1 and FoxO3, probably mediating survival by downregulating expression of the pro-apoptotic proteins NOXA and BIM. The stromal cell contact dependent survival of memory plasma cells in the bone marrow may serve as a paradigm for maintenance of tissue-resident memory cells in general, since inhibition of PI3K also ablates memory T and memory B cells of the bone marrow.

P.A4.02.04
IgG4-related disease of the skull base in two patients with normal serum IgG4
S. E. Detiger1, A. F. Karim2, J. van Loo2
1The Rotterdam Eye Hospital, Rotterdam, Netherlands, 2Departments of Internal Medicine and Immunology, section Clinical Immunology, Erasmus Medical Center, Rotterdam, Netherlands.

Introduction: IgG4-related disease (IgG4-RD) is an immune-mediated systemic fibro-inflammatory disease, which may mimic a variety of disorders. The pathogenesis is mostly unclear, but B cells, IgG4 positive plasma cells, and IgG4 antibodies, as well as the oligoclonal expansion of T cells seem to play an important role in the immunopathophysiology of IgG4-RD. IgG4-RD may manifest in almost every part of the human body. Here, we describe two patients with skull base manifestation of IgG4-RD that mimicked nasopharyngeal cancer. Case presentations: Patient 1, a 73-year-old male, with a history of smoking, diabetes mellitus type 1 and vascular disease, presented with a mass in the left nasopharynx extending to the cavernous sinus. Patient 2 was a 74-year-old male with a history of chronic obstructive pulmonary disease (COPD) and colon cancer who presented with a mass extending from the left nasopharynx into the inner ear with involvement of the left jaw joint. Both patients complained of pain and hearing loss. In both cases, serum IgG4 was normal and imaging did not show systemic manifestation of the disease. However, histology confirmed the diagnosis of IgG4-RD. Patients are currently treated successfully. Discussion: The described cases emphasize the broad clinical spectrum of IgG4-RD. The diagnostic workup may be challenging and serum IgG4 may be normal, as demonstrated in these cases. Careful histopathological examination of the tissues remains essential. Timely diagnosis of IgG4-RD is important to prevent secondary organ damage. Conclusion: IgG4-RD is a systemic disease and may present with normal serum IgG4.
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174

POSTER PRESENTATIONS

P.A4.02.05

T cells modulate B cell receptor signaling in mature antigen-naive B cells via CD40L


Survival of mature peripheral B lymphocytes requires B cell receptor (BCR) signaling in the absence of exogenous antigen-binding. However, the relationship between tonic BCR signals and antigen-triggered signals is currently unknown. Also, the mechanisms by which T-cell derived signals contribute to B cell survival remain unexplored. B-cell-specific overexpression of the BCR signaling molecule Bruton’s tyrosine kinase (BTK) induces spontaneous germinal center formation, autoantibodies and systemic autoimmunity. In aging BTK-transgenic mice naive B cells manifest increased IFNγ, IL-6, IL-10 and surface CD38 expression. Except for increased IL-10, this phenotype of naive B cells was dependent on B-T cell interaction, because it was lost in CD40L-deficient B-T-transgenic mice. To further investigate the role of CD40-CD40L interaction on B cell responsiveness, we compared BCR signaling in naive spleen B cells from CD40L-deficient and wild-type mice. We used intracellular flowcytometry to study phosphorylation of various BCR signaling proteins including PLCγ, as well as p56, which is downstream of the AKT pathway. Consistent with the in vivo pre-activated state of marginal zone (MZ) B cells, we found that in the absence of BCR stimulation the levels of phospho-PLCγ and phospho-p56 were higher in MZ B cells than in immature or follicular B cells. Hereby, no differences between CD40L-deficient and wild-type mice were observed. Upon BCR stimulation phospho-PLCγ and phospho-p56 increased in wild-type follicular B cells and particularly in MZ B cells, but these responses were lower in CD40L-deficient mice. Therefore, we conclude that CD40L signaling modulates BCR responsiveness in mature antigen-naive splenic B cells.

P.A4.02.06

Functional consequence of atypical B cells for in vivo development of antiviral B cell responses in patients with hemorrhagic fever with renal syndrome

P. F. Kerkman1, A. HäglinDemstedt1, J. Jangra1, A. Tuiskunen-Bäck1, J. Wigram Byström1, K. Malek2, J. Tauriainen1, J. Klingström1, K. Chandran1, A. Ahlm1, M. N. Farsøll;1
1-Umeå University, Umeå, Sweden; 2-‘Albert Einstein College of Medicine, New York, New York, United States; 3-Karolinska Institutet, Stockholm, Sweden.

Circulating B cells in healthy individuals comprise a small fraction of CD27-IgD-atypical B cells (ABCs). These cells may accumulate in patients with Systemic Lupus Erythematosus (SLE) nephritis. In vitro studies imply that ABCs are dysfunctional or exhausted but their in vivo biological function remains poorly understood. Hantavirus infections that cause hemorrhagic fever with renal syndrome (HFRS) lead to transient kidney dysfunction in patients, as shown by increased serum creatinine levels. We hypothesized that development of ABCs is associated with reduced kidney function, and that studies of HFRS could be used to assess if accumulation of ABCs is detrimental to the development of antiviral humoral immunity. Using longitudinal HFRS-patient samples stratified based on the median creatinine level, we demonstrate that ABCs accumulate in circulation of high creatinine patients but not in patients with lower serum creatinine levels. Phenotypical analysis showed that HFRS-induced ABCs have lower expression of activation markers and show reduced capacity for antigen presentation to T cells. Moreover, we found that ABCs have lower expression of co-stimulatory molecules, indicative of complement deposition. To assess the impact of ABCs on functional immune responses, we are characterizing longitudinal capacity of patients to mount antibodies that bind specifically and/or neutralize the homologous Hantavirus strains. Collectively, this study demonstrates an association between reduced kidney function and accumulation of ABCs in circulation. Moreover, our data shed light on the potential impact that accumulation of circulating ABCs may have on productive antiviral responses in HFRS-patients.

P.A4.02.08

A computational model of kinetic maturation in the germinal center

D. Lashgari1, M. Meyer-Hermann1, R. W. Sanders2, M. J. van Gils2, A. H. van Kamps3,4; 1-Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam, Netherlands, 2-Department of Systems Immunology and Braunschweig Integrated Centre of Systems Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany, 3-Department of Medical Microbiology, Academic Medical Center, Amsterdam, Netherlands.

Germinal centres (GC) are sites of affinity maturation, an evolutionary process in which B lymphocytes proliferate, undergo somatic hypermutations (SHM) and positive selection to produce high-affinity antibodies (Ab) eventually. Ab affinity (Koff) is defined as the ratio of kinetic constants koff and koff which determine the kinetics of the bond between B-cell receptors (BCR) and antigens (Ag) and between peptide-MHCs (pMHC) and T follicular helper (TFH) cells. We aim to investigate the dependency of spatiotemporal dynamics, affinity maturation, and output of the GC on individual contributions of kinetic constants. The model will be based on experimentally observed kinetic constants and affinities. We will extend a pre-existing agent-based model (ABM) of the GC (Meyer-Hermann et al., 2012) that comprises of the primary cellular mechanisms of the GC reaction (e.g., cell movement, B-cell proliferation, differentiation and apoptosis, SHM, Ag binding, positive B-cell selection). Binding kinetics will replace affinity representation in this model. We are currently implementing kinetics maturation in the ABM. We use three different scenarios to modify BCR-Ag binding kinetics since it is still not clear how koff and koff change during a GC reaction. It is assumed that koff is altered through SHM in all scenarios while koff is considered constant, affected by cell motility or affected only by SHM in separate scenarios. By comparing the output of these models to in-house generated experimental data and data from published studies, we aim to define the kinetics of GC reaction in more details.

References


P.A4.02.09

Multiscale modeling of plasma cell differentiation in germinal centers

E. Merino Tejero1, X. Gao2, A. P. Robert3, M. R. Martinez4, F. Crauste5; 1-Department of Medical Microbiology, Academic Medical Center, Amsterdam, Netherlands, 2-IBM Zurich Research Laboratory, Zurich, Swaziland, 3-Inria team Dracula, Lyon, France; 4-Division of Systems Biology, Karolinska Institutet, Stockholm, Sweden, 5-Department of Systems Immunology and Braunschweig Integrated Centre of Systems Biology, Helmholtz Centre for Infection Research; Institute for Biochemistry, Biotechnology and Bioinformatics, Technische Universität Braunschweig, Braunschweig, Germany.

Germinal centres (GCs) are sites of affinity maturation, an evolutionary process in which B lymphocytes proliferate, undergo somatic hypermutations (SHM) and positive selection to produce high-affinity antibodies (Ab) eventually. Ab affinity (Koff) is defined as the ratio of kinetic constants koff and koff which determine the kinetics of the bond between B-cell receptors (BCR) and antigens (Ag) and between peptide-MHCs (pMHC) and T follicular helper (TFH) cells. We aim to investigate the dependency of spatiotemporal dynamics, affinity maturation, and output of the GC on individual contributions of kinetic constants. The model will be based on experimentally observed kinetic constants and affinities. We will extend a pre-existing agent-based model (ABM) of the GC (Meyer-Hermann et al., 2012) that comprises of the primary cellular mechanisms of the GC reaction (e.g., cell movement, B-cell proliferation, differentiation and apoptosis, SHM, Ag binding, positive B-cell selection). Binding kinetics will replace affinity representation in this model. We are currently implementing kinetics maturation in the ABM. We use three different scenarios to modify BCR-Ag binding kinetics since it is still not clear how koff and koff change during a GC reaction. It is assumed that koff is altered through SHM in all scenarios while koff is considered constant, affected by cell motility or affected only by SHM in separate scenarios. By comparing the output of these models to in-house generated experimental data and data from published studies, we aim to define the kinetics of GC reaction in more details.

References


P.A4.02.10

Siglec-G deficiency leads to autoimmunity and earlier onset of chronic lymphatic leukemia

L. Özön, S. Mrozek, M. Korn, H. Fahnentiel, L. Nitschke; University of Erlangen, Erlangen, Germany.

Siglec-G is an inhibitory receptor on B cells. Siglec-G deficient mice show increased Ca2+ signalling particularly in B1 cells and a large B1 cell expansion. Furthermore, ageing Siglec-G deficient mice develop a lupus-like autoimmune disease. Higher activation of B cells as well as dendritic cells may contribute to this autoimmune disease. We have previously shown, that CD22 (Siglec-2) x Siglec-G double deficient mice develop a stronger autoimmune disease than Siglec-G deficient or CD22-deficient mice. One hypothesis to explain these data was that these two cell types contribute to this autoimmune disease. However, we could show in a new mouse model in which both Siglecs are defective in ligand binding, that this is not the case, as these mice do not develop autoimmune disease. Chronic lymphatic leukemia (CLL) is derived from CD5+ B cells. CD5+ B1a cells are largely expanded in Siglec-G deficient mice. We could show that these mice develop much earlier and stronger CLL in the Tcl1-transgenic mouse model.
POSTER PRESENTATIONS

P.A4.02.11
The Art2.2/P2X7-system differently affects functionality of murine CD4 T and iNKT subsets
G. Papadogianni, H. Georgiev, I. Ravens, G. Bernhardt; Hannover Medical School, Hannover, Germany.

Introduction: P2X7 represents an ATP-gated ion channel promoting inflammasome formation and T cell activation. P2X7 can be locked in an open state following ADP-ribosylation by the ecto-enzyme ART2.2 thereby triggering induction of apoptosis. We investigated the impact of the ART2.2/P2X7-system on functionality of iNKT and CD4 T cell subsets isolated from various organs.

Materials and Methods: Cell suspensions of thymus, spleen, Peyer’s Patches (PP) and peripheral lymph node (pLN) with or without prior immunization were prepared and incubated in presence or absence of the drug KN-62 blocking P2X7. Cells were then analyzed by flow cytometry to determine apoptosis and cytokine production.

Results: The expression patterns of P2X7 and ART2.2 correlated well with the observed degree of apoptosis. Whereas naive CD4 T cells resisted induction of apoptosis, follicular T cells were rather sensitive yet regulators were less affected than helpers. CD4 cell subsets of PP origin were more sensitive than their corresponding counterparts from pLN.

Moreover, thymic but not splenic iNKT cells resisted CD27 (activated/memory B cells) expression. The most prominent hits included type I interferons which strongly induced CD38. A similar cell differentiation state by high-throughput flow cytometry. By screening 2 donors we identified 10 B cell differentiation factors that altered surface IgG, induced CD38/CD138 essential but not sufficient for full human B cell differentiation.

B cell intrinsic TLR signaling is important for successful control of invading microbes via generation of pathogen specific antibodies and in promoting immune tolerance by generation of regulatory B cells. The aberrant activation of TLRs on B cells can lead to various disorders from primary immune deficiencies to autoimmunity. TLR stimulation alone is sufficient in driving B cell activation and metabolic reprogramming. However, it remains unresolved, which signaling pathway drives metabolic rewiring in these cells. To examine TLR-dependent metabolic reprogramming in B cells, we sorted naive (lgD-CD21hi) and memory (CD27+CD21lo) cells by and stimulated cells with the TLR9 agonist, CpG. Using a snapshot metabolomics and transcriptomics approach, we established that naive and memory human B cell subsets present distinct metabolic signatures following stimulation with CpG. To further examine metabolic changes in both B cell subsets, we monitored glycolysis and mitochondrial respiration by metabolic flux analysis. CpG was found to rapidly induced glycolysis in both subsets, whereas, mitochondrial respiration remained stable early after stimulation. Stimulation with CpG for 48 hours induced a marked enhancement of aerobic glycolysis and mitochondrial respiration in both B cell subsets. Early and late enhancements in glycolytic and mitochondrial respiration in naive B cells but not in memory B cells were primarily dependent on NF-kB signalling.

Lastly, blockade of metabolic reprogramming using IRIK1/2 inhibitors differentially impacted CpG induced cell activation, cytokine production, and early antibody production in both subsets. These results indicate that NF-kB dependent metabolic reprogramming and effector maturation are intricately linked in TLR stimulated B cells.

P.A4.02.14
Signalling requirements for metabolic reprogramming of human B cell subsets upon TLR stimulation
R. Steiner, G. Bantug, S. Wiedemann, C. Kunz, C. Hess; Univ. Hospital Basel, Basel, Switzerland.

B cell intrinsic TLR signaling is important for successful control of invading microbes via generation of pathogen specific antibodies and in promoting immune tolerance by generation of regulatory B cells. The aberrant activation of TLRs on B cells can lead to various disorders from primary immune deficiencies to autoimmunity. TLR stimulation alone is sufficient in driving B cell activation and metabolic reprogramming. However, it remains unresolved, which signaling pathway drives metabolic rewiring in these cells. To examine TLR-dependent metabolic reprogramming in B cells, we sorted naive (lgD-CD21hi) and memory (CD27+CD21lo) cells by and stimulated cells with the TLR9 agonist, CpG. Using a snapshot metabolomics and transcriptomics approach, we established that naive and memory human B cell subsets present distinct metabolic signatures following stimulation with CpG. To further examine metabolic changes in both B cell subsets, we monitored glycolysis and mitochondrial respiration by metabolic flux analysis. CpG was found to rapidly induced glycolysis in both subsets, whereas, mitochondrial respiration remained stable early after stimulation. Stimulation with CpG for 48 hours induced a marked enhancement of aerobic glycolysis and mitochondrial respiration in both B cell subsets. Early and late enhancements in glycolytic and mitochondrial respiration in naive B cells but not in memory B cells were primarily dependent on NF-kB signalling. Lastly, blockade of metabolic reprogramming using IRIK1/2 inhibitors differentially impacted CpG induced cell activation, cytokine production, and early antibody production in both subsets. These results indicate that NF-kB dependent metabolic reprogramming and effector maturation are intricately linked in TLR stimulated B cells.

P.A4.02.15
Elucidation of the in vitro requirements for the generation of plasma cells from human naive B cells
P. Unger1, T. Jorritsma1, M. Aalders1, A. ten Brinke1, T. Rispens1, M. van Ham1;1
1Sanquin Research, Department of Immunopathology, Amsterdam, The Netherlands, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands.

CD40 co-stimulation of B cells or CD40L expressing splenic T cells helps naive B cells, while Tfh cells, Tfh-like cells and Tfh cytokines are crucial for the generation of high-affinity antibodies. Whereas human memory B cells easily differentiate into antibody-secreting plasmablasts (CD27+CD38hi) upon CD40 and IL-21 stimulation in vitro, naive B cells do not. In vivo plasmablast differentiation occurs after repeated contact with Tfh cells in cyclic germinal centre reactions, but the mechanisms underlying these observations remain largely unknown. Progressive Tfh-like differentiation shows a switch from IL-21+ Tfh cells into IL-4+ Tfh-like cells in mice. Furthermore, CD40L expression on Tfh cells is subject to dynamic regulation. Here, we elucidated the minimal co-stimulation and cytokine requirements for human naive B cell differentiation into plasmablasts (PBs) and plasma cells (PCs). We investigated in vitro whether strength of CD40 co-stimulation in the presence of IL-4 and/or IL-21 regulates naive B cell differentiation into PBs and PCs and whether repeated co-stimulation is an intrinsic requirement for differentiation. Variation of CD40 stimulation by CD40L expressing 3T3 fibroblasts showed that strength of CD40 co-stimulation in presence of IL-21 was decisive for induction of the PB/PC transcriptional program (i.e. strong expression of Prdm1 and MIRA), while repeated co-stimulation was a key requirement to allow full effector PB and PC differentiation.

The elucidation of the requirements to induce human naive B cell differentiation into PCs in vitro now allow the investigation of the various steps in the human PC differentiation process and the discovery of targets to modulate desired and undesired antibody responses in vaccination, autoimmunity and allergy.

P.A4.02.16
Secretome screening reveals novel B cell differentiation factors
S. D. van Asten1,2, P. Unger1,2, S. Bliss1,2, T. Jorritsma1,2, C. Marsman1,2, N. Makazaji1,2, S. M. van Ham1,2, R. M. Spaapen1,2;1Dept. of Immunopathology, Sanquin Research, Amsterdam, Netherlands, 2Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands.

The most important function of B cells is the production of pathogen-specific antibodies. B cells become activated upon antigen encounter and may differentiate into memory B cells or antibody secreting plasma cells. Known pro-survival and (co-)activation signals include CD40L co-stimulation and the cytokines IL-4 and IL-21. Since these signals are essential but not sufficient for full human B cell differentiation in vitro, we set up a secretome screen to identify novel factors for B cell differentiation. We tested the differentiating capacity of 756 secreted proteins on human naive or memory B cells supported by CD40L expressing cells and suboptimal amounts of IL-21. After 9 days we determined the B cell differentiation state by high-throughput flow cytometry. By screening 2 donors we identified 10 B cell differentiation factors that altered surface IgG, induced CD38+CD138+ plasmablasts/plasma cells, and/or CD27+activated/memory B cells expression. The most prominent hits included type I interferons which strongly induced CD38+D. A similar phenomenon for type I interferons was previously reported in a different model system. Interestingly, both soluble FasL (sFasL) and MAP19 induced differentiation of B cells into plasmablasts and plasma cells. Moreover, sFASL and MAP19 increased the secretion of IgG1 and IgG4 isotypes, showing that both proteins augment the induction of functional plasma cells. Notably, FasL was upregulated on memory B cells upon CD40L interaction, suggesting that cognate sFasL-FasR is important for the specific differentiation into plasma cells. Thus this first secretome screen for B cell differentiation identified new regulators of B cell differentiation.
To induce antibody production, B cells internalize foreign antigen during infection or self-particles, like cell remnants, in auto-immunity and allimmunization after blood transfusion. This internalization requires antigen recognition by the B cell receptor (BCR) and is needed to present antigenic peptides via MHC II to attract the CD4+ T cell help required for class switching, somatic hypermutation and plasma cell differentiation. Often antigen is of a particulate nature. Although well described that B cells internalize particulate antigens, the underlying molecular pathways remain undefined. Using a high-throughput quantitative image analysis approach, we demonstrate that BCR-mediated signaling to PI3K promotes actin-driven particulate antigen acquisition and CD4+ T cell activation. According to current dogma, PI3K is recruited to the BCR via CD19, as part of the co-receptor. Strikingly, using the CRISPR-Cas9 technique, we demonstrate that CD19 is not required for BCR-mediated internalization of particulate antigen by human B cells. The redundancy in PI3K recruitment to facilitate particulate antigen internalization is mediated by the adaptor protein Nck. Human B cells thus employ the direct BCR-Nck-PI3K axis to modulate the actin cytoskeleton without clear CD19 co-receptor involvement for acquisition of particulate antigen. This knowledge may help to develop therapeutic agents to prevent auto- or alloantibody induction in auto-immune and transfusion in which internalization of large cell fragments containing self-antigen by B cells play a central role.

A splenic IgM memory subset harboring anti-bacterial specificities is sustained from persistent mucosal germinal center reactions.

To what extent immune responses against the gut flora are compartmentalized within mucosal lymphoid tissues in the absence of inflammation or external aggressions, remains a much-debated issue. We describe here, based on an inducible AID fate mapping mouse model, that systemic memory B cell subsets, including mainly IgM B cells, together with IgA B and plasma cells in the spleen, and IgA plasma cells in bone marrow, are generated in mice in the absence of deliberate immunization. While the IgA component appears dependent upon the gut flora, IgM memory B cells are still generated in germfree mice, albeit to a reduced extent and with reduced Ig gene diversification. Clonal relationships, renewal kinetics after anti-CD20 treatment, and BrdU labeling reveal that this long-lasting splenic population is to a large extent maintained by constant output of B cells clones persisting in Peyer’s patch and mesenteric lymph node germinal centers. IgM-secreting hybridomas established from splenic IgM memory B cells showed reactivity against gut luminal content, Gram+ and Gram- bacterial isolates and endogenous retroviruses. Ongoing activation of B cells in gut-associated lymphoid tissues thus generates a large systemic compartment showing long-lasting clonal persistence and harboring cross-reactive antigenic specificities endowing them with a protective capacity against systemic bacterial infections. This study reveals a new layer of protection achieved by diversified IgM memory B cells generated in homeostatic conditions.

The role of Krüppel-Like-Factor 2 (KLF2) transcription factor in plasma cell homeostasis

To unravel the role of KLF2 for PB/PC homeostasis, we are currently investigating KLF2-dependent target genes and signaling pathways by analyzing gene expression profiles of KLF2-deficient PBs and PCs in comparison to their wildtype counterparts. Furthermore, we are performing transfer experiments with KLF2-deficient PB/PC to analyze their migration and homing behavior.

This work was supported, in part, by the Deutsche Forschungsgemeinschaft (DFG) through research grant TRR130 (project P09).

Receptor signals in a MyD88 L265P mutation-driven murine model of diffuse large B cell lymphoma

Diffuse large B cell lymphoma (DLBCL) is one of the most abundant and aggressive tumors of the hematopoietic system and remains a clinical challenge. Especially, the activated B cell (ABC) subtype of DLBCL is characterized by a high relapse rate and poor five year survival of less than 40%. 30% of ABC DLBCL tumors have a recurrent leucine 265 to proline (L265P) mutation in the adaptor protein MyD88, which transmits signals of Toll-like receptors (TLR), the IL-1 receptor (IL-1R) and the BAFF/APRIL-sensing receptor TACI. MyD88 L265P stimulates lymphomagenesis by constitutively activating the transcription factor NF-κB. We showed previously, that the L265P mutant strongly binds wild type MyD88, which seeds signaling complexes of a high molecular weight, the so-called Myddosomes. Consequently, blocking of MyD88 heterodimerization sites killed L265P-mutated DLBCL cells better than wild type controls. Whether MyD88 L265P Myddosomes form spontaneously or are triggered by beforehand mentioned upstream receptors or in combination with B cell receptor (BCR) signals is not understood. In a novel mouse model, in which B cell specific expression of a wild type mouse orthologue to human MYD88 (L265P) causes an ABC DLBCL-like disease, we have begun to analyze in vitro the effects of stimulating or inhibiting TLRs, IL-1R and TACI on proliferation, cytokine secretion and Myddosome signaling. This is with a view to study the impact of these receptors on lymphomagenesis in vivo, which may add to the treatment of the many patients carrying the somatic MYD88 L265P mutation.

B cell specific interleukin 6 production plays a paradoxical role in B cell differentiation

B cells secrete interleukin 6 (IL-6) in response to TLR agonists or CD40L stimulation yet the significance of B cell specific IL-6 secretion in the progression of immune response is largely unknown. Here we show on isolated mouse splenic B cells that, among different TLR agonists, TLR4 agonist CpG induces the highest magnitude of IL-6 secretion which is antagonized by B cell receptor signaling. However IL-6 L6 B cells responded normally to CpG in terms of proliferation, prevention of activation induced cell death and activation marker expression in vitro. We then generated bone marrow chimeric mice by transferring bone marrow from B cell deficient mice mixed with bone marrow from either IL-6 KO or WT mice at 9:1 ratio and obtained B cell specific IL-6 mice and control mice respectively. Upon reconstitution, these mice were challenged with a T cell dependent antigen adjuvanted with either alum or TLR9 agonist CpG. We showed that B cell specific IL-6 deficiency resulted in weaker germinal center responses, lower number of antigens specific plasma cells, lower titers of specific antibodies and less antibody affinity maturation on antigen. Moreover, the same responses enhanced when antigen is adjuvanted with Cpg. These results suggest that, in the absence of CpG, B cell mediated IL-6 secretion is driven by B-T interactions and works in favor of T dependent responses. However, in the presence of CpG, B cell IL-6 production favors early commitment towards T independent plasma cell generation and thus plays role in dampening germinal center formation and affinity maturation.
Type I interferons (IFNs) are among the soluble mediators of anti-viral immune response. Although innate immune cells are responsible for the majority of type I IFN production in the body, recently, we showed that B cells, when stimulated with viral conjugated CpG-A type TLR9 agonists produce Type I-IFNs. However, other pathways that can induce Type I IFN in B cells and the overall significance of B cell specific Type I IFN production have yet to be characterized. Here, upon testing a range of stimulation conditions, we showed that dual stimulation of TLR3 and TLR4 is a potent inducer of type I IFNs in murine splenic B cells. Furthermore, when comparing WT and IFNAR KO mice, we identified that TLR9 induced upregulation of CD86 and CD317 on B cells are dependent on autocrine effects of IFNs. We then tested the extent to which B cell responses are mediated by IFNs by generating bone marrow chimeric mice using 1:1 ratio of WT and IFNAR KO bone marrow cells. Upon reconstitution, 1:1 ratio was conserved in total B cells and follicular cell compartments. However, majority of B1 and marginal zone cells came from WT mice while transitional cells showed IFNAR KO predominance indicating the differential effects of IFN signaling in B cell development. Once chimeric mice were infected with LCMV, we observed a significant increase in WT/IFNAR KO ratio in class switched B cells despite relatively unchanged ratios in germinal center and plasma cell compartments. These findings indicate that Type I IFNs play major roles at various stages of B cell development, activation and differentiation.

P.A4.03 Germinal centers and B cell differentiation - Part 3

P.A4.03.01
Comprehensive characterization of the human plasmablast response in dengue patients from India
C. Aggarwal1, K. Noyak2, M. Singla1, S. Gunisesty3, E. Reddy1, H. Panda1, G. Medigeshi2, R. Lodha1, S. Kabra2, R. Ahmed1, A. Chandler1, M. Kaja1
1International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India, 2All India Institute of Medical Sciences (AIIMS), New Delhi, India, 3Emory Vaccine Centre, ATLANTA, United States, 4Translational Health Science and Technology Institute (THSTI), Faridabad, India.

Humoral immune responses are thought to play a major role in dengue virus-induced immunopathology; however, little is known about the plasmablasts. Plasmablasts are terminally differentiated antibody secreting cells. The timing of their transient appearance in peripheral blood strikingly coincides with the presence of an infection /vaccination. One of a handful of studies from different parts of the world and none from India, has analyzed the B cell response in dengue infection. However, none of these studies were designed to comprehensively study the B cell response that includes phenotype and functionality. Here, we characterize the magnitude, specificity, and kinetics of the plasmablast response in acute febrile Dengue patients from New Delhi, India. We observe a massive plasmablast response which is heterogeneous and varies from 1% to 85% of B cells and averages at 22.5%. Longitudinal analysis showed that the plasmablasts can expand dramatically with frequencies increasing by more than 10-fold within a short window of 24-48hrs. A vast majority of these plasmablasts produces dengue specific IgG irrespective of whether the patient was primary or secondary infection. Also, the frequency of plasmablast was not significantly different in primary and secondary infection, but the rapidity of expansion was faster in secondary infection. This study is the first detailed analysis of the plasmablast response in Dengue patients in India and seeks to address whether plasmablasts are associated with clinical outcome and understand the biological mechanisms that determine the balance between protection versus pathology in dengue, which is critical for devising rational prevention/ control measures.

P.A4.03.02
Impact of type-1 and type-2 adjuvants on T follicular helper, T follicular regulatory and germinal center B cell populations
A. P. Basto1, A. Macera1,2,3, S. C. Almeida1,2, P. Campos1, F. Ribeiro1, S. Kumar1, M. Russo1, L. Graczyk1
1Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 2649-028 Lisboa, Portugal, 2Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal, 3Current affiliations: 3 - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, 4200-135 Porto, Portugal; and IBNAC – Instituto de Biologia Celular e Molecular, Universidade do Porto, 4200-135 Porto, Portugal, 4Current affiliation: Núcleo de Doenças Infecciosas (NDD), Universidade Federal do Espírito Santo (UFES), Vitória - ES, 29040-107, Brazil, 5Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo - SP, CEP 05580-900, Brazil.

The generation of antibody responses is crucial for the protective immunity induced by most effective vaccines. The efficacy of those vaccines relies not only on the magnitude but also on the persistence, affinity and specificity of the antibodies elicited. These qualitative properties of the humoral immune response depend on appropriate germinal center (GC) reactions, which are supported by T follicular helper (Tfh) cells and regulated by T follicular regulatory (Tfr) cells. Although it is known that different adjuvants induce distinct subclasses of antibodies, their influence on GC reactions is yet poorly understood. Here we investigated the impact of a panel of type-1 and type-2 adjuvants on Tfh, Tfr and GC B cell populations. Mice were immunized with the same model antigen (ova/rolling) in combination with Th2-polarizing adjuvants (potassium alum or incomplete Freund’s adjuvant) or with three different formulations containing a toll-like receptor (TLR) ligand with Th1-polarizing properties (CpG oligodeoxynucleotides). As expected, Th2-type adjuvants induced an IgG1 class-switch whether formulations containing the TLR9 ligand induced higher IgG2a responses. We found that Th2-adjuvants were more effective in inducing proliferation of GC B cells (CD19+ Fas−GL7+) but this effect could not be ascribed to quantitative differences in Tfr or Tfr populations, since CD4+CXCR5+PD1+ cell levels and Tfr: Tfh ratios did not correlate with GC B cell expansion. Similar conclusions were obtained from Th1-prone (C57BL/6) and Th2-prone (Balb/c) mouse strains. Qualitative differences of Tfh populations generated under Th1- versus Th2-polarizing conditions are now being evaluated through differential gene expression analysis.

P.A4.03.03
Phenotypical and functional characterization of IL-10-producing regulatory B cell subsets
V. Duong1, N. Farimany1, N. Figer2, K. Lee3
1Inflammation Research Group, Institute of Clinical Chemistry,Hannover Medical School, Hannover, Germany.

Recent studies have recognized that B cells can negatively regulate pathogenic T cell responses in an antibody-independent manner, which has led to the concept of regulatory B cells. Regulatory B cells have been functionally defined in mice and humans by their ability to produce and inhibit the anti-inflammatory cytokine IL-10 and have been suggested to exhibit immunosuppressive function in the context of autoimmune and inflammatory disease. However, the nature and origin of regulatory B cells is controversial and it is still unclear whether they represent a distinct B cell lineage or a dynamic cellular state. Our study reveals that regulatory B cells are not a predetermined novel B cell lineage, but are formed by a pool of innate-like ‘classical’ B cells. Our data show that the majority of IL-10-dependent regulatory B cell subsets resides within both B1 and B2 B cell compartments and originate from B1a and marginal zone B cells. We directly demonstrate that these IL-10-producing B cell subsets exhibit suppressive function on the production of proinflammatory cytokines by CD4+ and CD8+ T cells. Moreover, these regulatory B cells exhibit a high degree of self-reactivity and produce autoreactive antibodies directed against dsDNA and ssDNA. Our study further suggests that regulatory B cell differentiation is guided by BCR-responsiveness and signaling thresholds. Thus, regulatory B cells act as an immunological double-edged sword: they exhibit potent immunoregulatory activity on inflammatory T cell responses, but, owing to a high degree of self-reactivity, can also turn into harmful autoantibody-producing plasma cells.

P.A4.03.04
Dissecting the T cell-extrinsic requirements for Tfh cell differentiation
J. Huber1, D. Baumann2;2,3
1Institute for Immunology, Planegg-Martinsried, Germany.

T follicular helper (Tfh) cells represent the primary CD4+ T cell subset that provides crucial help to B cells for potent antibody responses during infection and vaccination. While there is emerging evidence for the molecular mechanisms in T cells that drive Tfh cell differentiation, e.g. up-regulation of the Tfh cell-associated transcription factor Bcl6 and down-regulation of the Bc6 antagonist Blimp-1, the Tfh cell-extrinsic signals that induce the Tfh cell fate remain largely unknown. Dendritic cells (DCs) are potent inducers of the Tfh cell phenotype and this induction is independent of cognate interactions with B cells. Thus, DC-derived signals seem to instruct molecular programs in responding T cells. While a differential role of splenic CD8+ and CD8+ DC subsets has been reported for the activation of CD8+ or CD4+ T cells, respectively, less is known about the DC subset(s) that induces(s) Tfh cells and with which co-stimulatory networks, but co-stimulatory pathways are involved in the DC-Tfh cell interactions that drive Tfh cell fate decisions. Here, we tested how the Bcl6/Blimp-1 axis in DCs impacted their ability to induce Tfh cells. These data provide new insights into the mechanisms by which DCs induce Tfh cells and shape their identity.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Approximately 15% of IgG antibodies acquire glycans in the Fab domain on consensus amino acid motifs (Asn-X-Thr/Ser, X ≠ Pro), so-called N-glycosylated Fab sites. Once acquired, Fab glycans can affect antibody stability and affinity. The human naïve B cell repertoire is largely devoid of N-glycosylated Fab sites with only a few germline-encoded alleles displaying such sites. IgM/Fab IS-34 is such an allele and besides having a germline-encoded N-glycosylation site (N57) it is also one of the more frequently used human Vα alleles. Nonetheless, they can cause non-glycosylated Fab sites. It is most likely due to the surrounding amino acid micro-environment. It has been shown that charge and hydrophobicity of the core amino acid X of the consensus motif plays an important role in likelihood of glycosylation. Interestingly, the germline N57 core amino acid X is histidine which was shown to be favorable for glycosylation.

The question whether neighboring amino acids can also influence glycosylation status of the consensus motif. Furthermore, other VH family members have the potential to gain this site in the CDR2 upon somatic hypermutation. By introducing the N57 glycosylation site in multiple VH family members we can investigate its glycosylation status in the context of different neighboring amino acids. Thereby, we intend to demonstrate the contribution of adjacent core amino acids to glycosylation status of the canonical or potential SHM induced N57 glycosylation site in VH4 gene family members.

Modeling transcriptionics of T follicular helper cells across different strains of mice.

A. L. Lemarquardt1,2, F. P. Theodoris1, S. H. Lund1, J. Jenndotti1, H. K. Einarsson1, B. R. Ludvigsson1,2
1University Hospital of Iceland, Reykjavik, Iceland, 2Faculty of Medicine, University of Iceland, Reykjavik, Iceland, 4University of Iceland, Reykjavik, Iceland.

Selective IgA deficiency (IgAD) is the most common primary antibody deficiency in Caucasians with affected individuals suffering from an increased burden of autoimmunity with autoantibody positivity, atopy and infections. Our ex vivo analysis of lymphocyte populations in IgAD shows B cell defects with significantly lower numbers of transitional B cells (CD19+CD24+CD38hi) and class-switched memory B cells (CD20+CD27hi). A novel T cell independent defect was seen in vitro after CpG (ODN-2006) stimulation where it failed to induce IgA production and enhanced the defect in transitional B cells, especially within B regulatory cells expressing IL-10. Proportions of T cell populations ex vivo as well as in vitro induced T effector cells and T regulatory cells remained however normal. The signalling analysis of ERK was normal after CpG while pSTAT3 showed decreased signal in both total T and B cells following IL-21 stimulation. In serum from IgAD individuals a raised concentration of sCD40L was seen without stimulation, correlating inversely with IgA in IgAD but not IgG and IgM.

Pathway enrichment analysis of mRNA transcriptionics of isolated B cells from IgAD individuals before and after CpG stimulation points towards defects related to longevity and survival. Collectively, our data indicates a complex B cell defect with defects in both T cell dependent and independent responses and overproduction of CD40L pointing towards a compensatory B cell overstimulation which may be important to assess in the treatment with “personalised immunotherapies” of individuals affected by IgAD and concomitant immunodepression.

The mutation patterns in B-cell immunoglobulin receptors reflect the influence of selection acting at multiple time-scales

Y. Louzoun1, G. Haro1, s. Klein1,2
1Bar Ilan, Ramat Gan, Israel, 2Yale, New Haven, United States.

During the adaptive immune response, B cells undergo a process of clonal expansion, somatic hypermutation of the immunoglobulin (Ig) genes and affinity-dependent selection. Over a lifetime, each B cell may participate in multiple rounds of affinity maturation as part of different immune responses. These two time-scales for selection are apparent in the structure of B-cell lineage trees, which often contain a trunk consisting of mutations that are shared across all members of a clone, and several branches that form a canopy. These patterns are shared by a subset of clone members. The influence of affinity maturation on the B-cell population can be inferred through the pattern of somatic mutations in the Ig. While global analysis of B-cell lineage trees has shown evidence of strong selection pressures shaping the B-cell population, the effect of different time-scales of selection and diversification has not yet been studied. Analysis of B cells from blood samples of three healthy individuals identifies a range of clone sizes with lineage trees that can contain long trunks and canopies. We here show that observed mutation patterns in the framework regions (FWRs) are determined by an almost purely purifying selection on both short and long time-scales. By contrast, complementarity determining regions (CDRs) are affected by a combination of purifying and antigen-driven positive selection on the short term, which leads to a net positive selection in the long term. In both the FWRs and CDRs, long-term selection is strongly dependent on the heavy chain variable gene family.

Converging Evolution Leads to Near Maximal junction diversity through parallel mechanisms in B and T cell receptors

Y. Louzoun, J. Benichou; Bar Ilan, Ramat Gan, Israel.

The T and B cell receptor (TCR and BCR) complementarity determining region 3 (CDR3) genetic diversity is produced through multiple diversification and selection stages. Potential holes in the transcriptional CDR3 repertoire emerge. In order to address the genetic diversity of the adaptive immune system, appropriate quantitative measures for diversity and large scale sequencing are required. Such diversity should incorporate the complex diversification mechanisms of the adaptive immune response and the BCR and TCR loci structure. We combined large-scale sequencing and diversity measures to show that TCRs have a near maximal CDR3 genetic diversity. Specifically, TCR have a larger junctional and V germline diversity, which starts more 5’ in VH than BCs. Selection decreases the TCR repertoire diversity, but does not affect BCR repertoire. As a result, TCR is as diverse as BCR repertoire, with a biased CDR3 length toward short TCRs and long BCRs. These differences suggest parallel converging evolutionary tracks to reach the required diversity to avoid holes in the CDR3 repertoire.

Inflammation mediated by T-bet during blood-stage infection modulates the development of humoral immunity to malaria

A. Ly1, L. Yang1, W. Shi1, V. Ray-Carneiro1, J. I. Goodman1, K. L. Jacobson1, G. T. Belz2, A. Kallies1,2, D. S. Hansen1,2
1The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, 2The University of Melbourne, Department of Medical Biology, Parkville, Australia, 3Monash University, Department of Biochemistry and Molecular Biology, Clayton, Australia, 4The University of Melbourne, Peter Doherty Institute for Infection and Immunity, Parkville, Australia, 5The University of Melbourne, Department of Microbiology and Immunology, Parkville, Australia.

Malaria is a preventable but serious infectious disease that burdens developing countries, in part because natural immunity to the malaria-causing Plasmodium parasites takes years of repeated infections to develop. The reasons for this are elusive; however, growing immuno-epidemiological evidence indicate that parasite-specific antibodies and memory B cells (MBCs) that mediate protection, are poorly generated and short-lived in endemic areas. In contrast, MBCs can be induced effectively in low transmission settings, suggesting that acute infection may undermine the acquisition of humoral immunity. Using preclinical model of malaria, we demonstrated that infection-associated pro-inflammatory pathways hinder germinal centre (GC) and MBC development. To delineate the underlying molecular mechanisms, GC responses to blood-stage P. berghei ANKA was examined in mice deficient in the pro-inflammatory, T helper 1 transcription factor, T-bet. Genetic ablation of T-bet in CD4 T cells significantly improved the differentiation rates of T follicular helper cells, consequently boosting GC and antibody responses to infection.

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T-bet not only affected T cell help, but also modulated intrinsic B cell activity. Deletion of T-bet in B cells enhanced the GC response to infection, and altered its dark and light zone dynamics, critical for high-affinity antibody production. RNA sequencing further revealed that T-bet significantly modulated the GC B cell transcriptional program during infection. We show that inflammation driven by T-bet during blood-stage malaria suppresses the acquisition of humoral immunity, by compromising T cell help and GC B cell differentiation. This knowledge will inform next generation vaccine and therapeutic strategies to elicit sustained protection against disease.

P.A.A.03.12
Human Antibody Transgenic Rabbits
S. Offner, F. Ros, E. Köninger, S. Klosterrmann, H. Niersbach; Roche Diagnostics GmbH, Penzberg, Germany.

Antibodies generated in animal hosts make up the larger part of marketed therapeutic antibodies. Their generation undergoes positive and negative selection as well as affinity maturation in vivo, which lead to antibodies with robust therapeutic properties. Rabbits use a distinct diversity generation mechanism, which has been exploited since long to achieve very high specificity and affinity. However, antibodies derived from wild type animals such as mouse, hamster, rat or rabbit still have to undergo a process called “humanization” before being used as therapeutics in humans, limiting flexibility and adding time to the drug development process. Here, we report the development of rabbits transgenic for human immunoglobulin genes and the characterization of their B-cell compartment. Highly affinity antibody candidates were yielded from these human antibody transgenic rabbits showing an excellent “humanness” and a specific therapeutic mode of action.

P.A.A.03.13
Role of dendritic cells in antigen transport, transfer and B cell activation

Dendritic cells (DCs) are phagocytic cells, which sample antigen (Ag) in the periphery and migrate to the lymph node (LN) where they activate T cells and potentially B cells. Previously, we reported that DCs can transfer Ag to B cells after an extracellular release that was called “regurgitation”. The modes of B cell activation by DCs and the underlying mechanisms still remain unknown. We have investigated: 1) Ag transport by DCs and distribution in the LN, 2) the role of DCs in B-cell activation in vivo and in co-culture in vitro, 3) the mechanisms of early B cell activation by analysing NF-κB pathway in B cells. Ag carrying DCs migrated to LN within 18h after footpad mice injection allowing to Ag colocalization with both CD11b+ and CD8α+ resident DCs. At 48h, we observed proximal positioning of Ag with follicular B cells. At 72h, Ag-loaded DCs were able to induce B cell activation and differentiation in vivo. In co-culture in vitro, Ag-loaded DCs activated anti-HEL B cells and importantly Ag release in DC supernatant by regurgitation induced also early B cell activation. BCR stimulation with specific antigen and Ag released by DCs were able to activate NF-κB in particular subunit nuclear mobilization. DCs are an important cell transporter of native Ag from the periphery and also activators of B cells in vivo and in co-cultures in vitro. We expect to provide new insights into Ag encounter by B cells in vivo, and novel approaches of DC targeting to elicit humoral immunity.

P.A.A.04.14
CLASS SWITCH RECOMBINATION DEFICIENCIES MOROCCAN CASES SERIES
H. OUAZ1, I. Benhassen1, J. El Bakkouri2, L. Jedda3, H. Salih4, A. Bousfiha1, F. Alaour2.
1-Biology Laboratory and Health/ Immune and Metabolic Pathology Team- Faculty of Sciences Ben M’Sik. Hassan II University, casablanca, Morocco, 2-University Hospital Casablanca- Centre for Chronic Immunodeficiency (CCI), University Medical Centre Freiburg, Freiburg, Germany, 3-Integrated Research Training Group (IRTG) Medical Epigenetics, Collaborative Research Centre 992, Freiburg, Germany, 4-Faculty of Biology, Albert-Ludwigs-University of Freiburg, Freiburg, Germany.

Class Switch Recombination Deficiencies (CSR-D) is characterized by a decrease serum IgG and IgG with normal or increased IgM. Several genetic mutations were defined that affects the interaction between T and B cells required to produce IgA and G. There are two main forms: X-linked and autosomal recessive. Clinical manifestations are dominated by recurrent infections (pulmonary, ENT, digestive…), especially with opportunistic pathogens. Our study reports 17 cases of hyper IgM followed in our department since 1995. There are 8 boys and 9 girls with 12 patients reporting a parental consanguinity. The mean age at diagnosis is 4.5 years (5 months to 12 years). The clinical manifestations were dominated by respiratory infections (13 cases), including 6 cases of bronchiectasis and two CMV pneumonia. Chronic diarrhea was observed in 4 patients with cryptosporidiosis. ENT infections were noted in 6 cases. One patient had three episodes of meningitis and another one BCGitis. All patients had low IgG and IgG and 11 patients had high levels of IgM. Five patients had thrombocytopenia. Unlike Europe, where X-linked hyper-IgM syndrome (HIGM1) is the most common, this Moroccan series shows the highest of autosomal recessive forms in our context as suggested by the inbreeding rate, the frequency of female and the predominance of phenotypes with lymphoproliferation.

P.A.A.04.15
Transcriptional dysregulation of CVID patients harboring C104R TNFRSF13B mutation
N. J. Ramirez1,2,3, M. E. R. Schmitt4, M. Walter5, M. L. H. Henrichs1, G. H. Winkelmann6, J. K. H. Haukasi1, C. Bossen1, B. Grimbacher1,2,3.
1-Institute of Cellular and Molecular Immunology, Georg August University, Göttingen, Germany, 2-Institute of Cellular and Molecular Immunology, University Hospital Casablanca - Centre for Chronic Immunodeficiency (CCI), University Medical Centre Freiburg, Freiburg, Germany, 3-Integrated Research Training Group (IRTG) Medical Epigenetics, Collaborative Research Centre 992, Freiburg, Germany, 4-Faculty of Biology, Albert-Ludwigs-University of Freiburg, Freiburg, Germany.

The process of affinity maturation in Germline Centres is critical for the production of efficient antibodies and recall responses against pathogens. The dynamics of B cell movement, proliferation, selection and thus affinity maturation inside Germline Centres can be successfully captured by mathematical modelling. These models rely on a abstract representation of protein-protein interactions to translate the effects of somatic hypermutation into a change in affinity to the antigen. In light of recent challenges for the development of broadly neutralizing vaccines, it would be of interest to study the effect of multiple antigens or epitopes driving Germinal Centre dynamics. The sequence representation used in silico so far has structural biases and potential effects onto the emerging cross-reactivity of receptors produced by the Germinal Centres. Here, we compare the consequences of different sequences of representations and the consequences on the properties of affinity maturation. The results help to understand the efficiency of different vaccination regimens, and to solve emerging controversies regarding the vaccination strategies using cocktails of antigens at the same time or following sequential immunizations.

P.A.A.04.16
Sequence representation in Germline Centre simulations
P. A. Robert1, M. Meyer-Hermann1.
1-Biology Laboratory and Health/ Immune and Metabolic Pathology Team- Faculty of Sciences Ben M’Sik. Hassan II University, casablanca, Morocco, 2-University Hospital Casablanca- Centre for Chronic Immunodeficiency (CCI), University Medical Centre Freiburg, Freiburg, Germany.

The treatment of common variable immunodeficiency (CVID) involves immunoglobulins of the IgE isotype are associated with allergic inflammation and immunity against helminths. Affinity-matured IgE-producing plasma cells (PCs) are thought to be mainly generated by sequential class switch recombination via an IgG1 intermediate step. However, the generation and homeostasis of IgE-producing plasma cells (PC) is still poorly understood.

P.A.A.04.17
The immunoglobulin tail tyrosine motif in transmembrane IgE is required for generation and/or survival of IgE-producing plasma cells
M. E. R. Schmitt1, N. Engel2, J. Wieroncl2, D. Vöhringer2.
1-Department of Infection Biology, Institute for Clinical Microbiology, Immunology and Hygiene, University Hospital and Friedrich-Alexander University (FAU), Erlangen-Nürnberg, Germany, 2-Institute of Cellular and Molecular Immunology, Georg August University, Göttingen, Germany.

Introduction: Immunoglobulins of the IgE isotype are associated with allergic inflammation and immunity against helminths. Affinity-matured IgE-producing plasma cells (PCs) are thought to be mainly generated by sequential class switch recombination via an IgG1 intermediate step. However, the generation and homeostasis of IgE-producing plasma cells (PC) is still poorly understood.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

POSTER PRESENTATIONS

Objectives and Methods: To investigate the cytoplasmonic tail of mIgG1 or mIgG is important for the IgG response we analysed mutant mice after infection with the helminth Angiostrongylus brasiliensis. We compared IgG1YF and IgEYF mice in which the immunoglobulin tail tyrosine (ITT) motif in mIgG1 or mIgE is inactive. In addition we used IgEYF mice with a truncated cytoplasmonic Ig tail.

Results: Our results show a normal IgG memory response in IgG1YF mice. In contrast, IgE+ PCs in IgEYF mice were reduced after secondary infection. In IgEYF mice the IgE+ PCs were diminished after first and second infection. Serum IgG levels were reduced in both IgG mutant mice.

Conclusion: Our results show that the ITT motif in mIgG1 is important for the IgG, but not the IgG memory response. However the ITT motif in mIgE and the intracellular part of IgG are important for the IgE response. This led to the hypothesis that the ITT motif in mIgG1 is important for the generation or survival of IgE+ PCs and we will now investigate whether this hypothesis can be validated.

P.A4.03.18
Hypermunization with RhD generates a broad and persistent repertoire of IgM and IgG antigen-specific B cells
M. Berkowsk1, N. van der Bolt2, L. della Valle1, H. Uspeert2, O. Verhagen1, M. van der Burg2, A. ten Brinke3, M. van Ham3, G. Vidarsson1, E. van der Schoor2; 1Sanquin Research, Amsterdam, Netherlands, 2Leiden UMC, Leiden, Netherlands.

RhD-negative women, pregnant with a RhD-positive child, receive anti-D immunoglobulins (Ig) from hypermunized donors to prevent alloimmunization. The characteristics of anti-D-Igs and evolution of the anti-D response remain poorly characterized.

RhD-specific CD27+IgM+, CD27-IgM+ and CD27-IgG- B-cells, equally distributed between IgM+ and IgG+, were identified in all donors. Rearranged Ig genes in RhD-specific B-cells carried somatic hypermutations, displayed long IgH-CDR3s and showed a broad repertoire skewed towards IGHV4-34, IGHV3-3 and IGHV3-30 genes. Frequently utilized IGHV3-34 genes showed limited selection for replacement mutations in IGH-CDRs, implicating structural advantage in RhR recognition. ~30% of identified anti-D rearrangements represented clonally related cells. The majority of clones spanned either the IgM+ or IgG+ B-cell subsets and clones can have life-spans of at least 10 years. Remarkably, recent time point B cells harbored less mutations than their clonal relatives at earlier time points and the somatic hypermutations rate does not increase over time.

In conclusion, the RhD response involves a broad, but restricted Ig gene repertoire, persisting over time. IgM+ and IgG+ B-cells are important for the maintenance of a lasting allomunization response and recruitment of IgM+ memory B-cells plays an important role in the memory response.

P.A4.03.19
Identification of follicular dendritic cells (FDCs) from human tonsils and binding of model complex complement components
L. Raleigh, E. Lekova, M. SAISANA, K. Nistala, C. Elison; Glaxosmithkline R&D, Stevenage, United Kingdom.

Follicular dendritic cells (FDCs) are a cell type found in the germinal centre. Complement-opsonised antigen can traffic to the germinal centre where antigen is then colocalised with FDCs. FDCs are the only known cell type to retain whole native antigen. This intact antigen is re-presented on the FDC cell surface for periodic interaction with B cells, aiding antibody affinity maturation. This complement-binding function of FDCs has relevance to numerous diseases, including autoimmunity and HIV infection.

Published work using primary human FDCs is limited as they are low in frequency and difficult to isolate. Here we describe a method we have developed to isolate FDCs from human tonsil, obtaining ~1000-3000 FDCs per donor. We have confirmed by flow cytometry, cell sorting and imaging of cell surface markers that these cells are indeed FDCs. We have found that these FDCs express the complement receptor 1 (CD35) and a subset also express complement receptor 2 (CD21). Through the antigen opsonisation process, C3 fragments are cleaved to leave C3dg which can bind to CD21 with high affinity. We have built a fluorescent model antigen incorporating C3dg and have generated robust data demonstrating binding of these complexes to FDCs. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.

P.A4.03.20
MicroRNA-155 is essential for the proliferation and survival of plasmablast B-cells
R. Leyland1, G. Arbore1, L. Biggin2, T. Henley3, S. Andrews2, R. Brink1, E. Vigorito1, M. Turner2; 1Sheffield Hallam University, Sheffield, United Kingdom, 2Abraham Institute, Cambridge, United Kingdom, 3Garvan Institute of Medical Research, Sydney, Australia.

The differentiation of B cells after antigen exposure is essential for antibody production and clearance of invading pathogens. During the early B-cell response to T-cell deficient antigen, B-cells differentiate into B-cell blasts, plasmablasts and germinal centre cells. Plasmablast B-cells secrete unmaturated low affinity antibodies and typically differentiate into short-lived plasma cells. Although this response is short-lived, it can be critical for neutralisation of rapidly dividing pathogens. The posttranscriptional regulator microRNA-155 (miR-155) has been shown to be critical for the germinal centre response; however, the cellular and molecular mechanisms by which miR-155 regulates the plasmablast response are not well understood. We utilized SW+/- mice, either sufficient or deficient for miR-155, to assess the contribution of miR-155 to the plasmablast response after immunisation with hen egg lysozyme (HEL) conjugated to sheep red blood cells (HEL-SRBCs). We show that miR-155 is required to sustain the extrafollicular plasmablast response and is essential for plasmablast survival and proliferation. MiR-155 deficient, HEL-specific plasmablast B-cells showed an increase in apoptosis and defects in cell cycle progression and DNA replication compared to wild type controls. Therapy to transcriptional control of miR-155 sufficient and deficient SW+/- B-cells we determined that miR-155 indirectly regulates genes involved in cellular processes such as the DNA metabolic process, DNA nucleosome assembly, DNA replication initiation and the mitotic cell cycle process. Overall, our data demonstrate a complex mechanism of plasmablast regulation by a single microRNA, which provides new insight into antibody production during the early response to infection and vaccination.

P.A4.03.22
B cell development sans BCR responsiveness due to unfolded protein response triggered MeF2c protein degradation
Y. Chen1, Y. Su2, J. T. Kung2; 1Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, 2National Laboratory Animal Center, National Applied Research Laboratories, Taipei, Taiwan.

BCR engagement leads to activation and clonal expansion of B cells. The I-A<sup>2</sup>B<sup>-</sup> mutant mouse possesses a branch site point mutation in the H2-2a gene that causes highly reduced I-Aa protein expression. As I-A+ is a heterodimer made up of I-Aa and I-Ab, BCR responsiveness was restored by transduced I-Aa expression and by Bif, the UPR sensor. Reducing the load of unpaired I-Ab also restored BCR responsiveness of I-A<sup>2</sup>B<sup>-</sup> B-cells. MeF2c protein, a transcription factor required for BCR-stimulated proliferation, was missing in I-A<sup>2</sup>B<sup>-</sup> and that transduced MeF2c expression restored BCR responsiveness. MeF2c protein appeared in I-A<sup>2</sup>B<sup>-</sup> B-cells after addition of proteosome inhibitors. MeF2c degradation was mediated by Skp2 E3 ligase and that knock-down of Skp2 mRNA in I-A<sup>2</sup>B<sup>-</sup> B-cells restored BCR responsiveness. Our results point to a generalized incompatibility between BCR responsiveness and increased Skp2 stability. They also imply the existence of regulatory mechanisms other than Ig gene rearrangement that govern MeF2c turnover in a specific, exquisite, and dynamic fashion.

P.A5.01 Initiation of immune responses - Part 1

P.A5.01.01
XCR1<sup>+</sup> Dendritic Cells are required for intestinal Th1 homeostasis
F. Ahmadz1, K. Müller Lüd1, B. Mollerstein1, W. W Apage1,2; 1Biomedical centre, Immunology section, Lund, Sweden, 2Centre d’Immunologie de Marseille-Luminy, Aix Marseille Université, INSERM, CNRS UMR, Marseille, France, 3Division of Immunology and Vaccinology, National Veterinary Institute, Technical University of Denmark (DTU), Kongens Lyngby, Denmark.

The intestinal mucosa contains several classical dendritic cell (cDC) subsets each of which appear to play a non-redundant role in intestinal T cell homeostasis. Intestinal cDC1, similar to cDC1 in other tissues, are dependent on the transcription factor IRF8 for their development. Utilizing CD11c-cre.IRF8<sup>fl/fl</sup> mice, we have recently provided data to suggest that intestinal cDC1 are important for intestinal Th1 homeostasis and priming (1). CD11c+ is however expressed by multiple cell types, including intestinal macrophages, plasmacytoid DC and subsets of B and T cells. To determine whether intestinal Th1 responses are dependent on cDC1 we have generated XCR1<sup>-</sup>Cre.IRF8<sup>fl/fl</sup> mice, that specifically and exclusively lack cDC1. Similar to CD11c-cre.IRF8<sup>fl/fl</sup> mice, XCR1<sup>-</sup>Cre.IRF8<sup>fl/fl</sup> mice lack Th1 cells in the small intestine and enterocolitis. Similar to CD11c<sup>-</sup> and displayed markedly reduced numbers of Th1 cells in the colon. In preliminary experiments, we have observed that intestinal Th1 numbers are normal in IL-12p35 or IFN-α receptor (IFNAR) deficient mice indicating that cDC1 driven intestinal Th1 homeostasis is independent of IL-12 and interferon signalling. Current studies are thus focused on identifying the key cDC1 derived factors required for intestinal Th1 homeostasis as well as the environmental triggers that promote Th1 development in the intestine.

1. Luda et al., IRF8 Transcription-Factor-Dependent Classical Dendritic Cells Are Essential for Intestinal T Cell Homeostasis, 2016, IMMUNITY, 44, 860-874.
POSTER PRESENTATIONS

P.A5.01.02
Toll-like receptor-dependent activation of dendritic cells in cattle

G. T. Barut1,2, C. C. Talecker3, A. Sommerfeld4
1Institute of Virology and Immunology, Bern and Mittelhäusern, Switzerland, 2Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, 3Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland.

Introduction: Signaling via toll-like receptors (TLR) mediates a potent activation and maturation of dendritic cells (DC). Therefore, knowledge on TLR ligand responsiveness, which differs both with DC subset and species, is of major interest for vaccine adjuvant development. Method: Two methods to determine bovine DC activation after stimulation of BMDMs with TLR ligands Gdsriquimod, LPS, and Pam3CSK4 were compared: 1) Lipofuscin as a surrogate marker of newly synthesized material; 2) the expression of cytokine receptor 7 (CCR7) as determined by flow-cytometry after 4h stimulation, and 2) detection of phosphorylated p38 mitogen-activated protein kinase (MAPK) by phospho-flow after 15min. Results: After 4h stimulation of BMDMs, all DC subsets upregulated CCR7 irrespective of the ligand used, which contradicted substuctspecific TLR expression in cattle. By contrast, early detection of phosphorylated p38 in response to TLR ligands correlated with TLR gene expression. In plasmacytoid DC, only Gardiquimod triggered p38 phosphorylation. As expected from TLR2 expression data, both conventional and plasmacytoid DC subsets were activated by Pam3CSK4. In contrast, none of the DC subsets, or only monocytes responded to LPS, with increased p38 phosphorylation, again corresponding to the low TLR4 expression on blood DC of cattle. Conclusions: Bystander effects in BMDM cultures have a strong impact on TLR ligand responsiveness of DC subsets when CCR7 is analyzed after 4h. In contrast, flow cytometric detection of p38 phosphorylation in PBMC is a sensitive method to screen for functional and subset-specific TLR expression. This project was supported by the European Union's Horizon 2020 Program under Grant Agreement 633184.

P.A5.01.03
The impact of vaccine-induced innate signals on breadth and function of CD8 T cell responses

C. A. Bernhard, N. Lausterbach, T. Brocker

Dendritic cells (DCs) are considered the most potent antigen-presenting cells (APCs), which directly prime or cross-prime MHC class I-restricted cytotoxic T lymphocytes (CTLs). In the context of vaccination with recombinant adenovector vaccines (rAd), we could previously demonstrate that also vaccine-infected CD16+ and -SIGNR1+ macrophages were sufficient to prime broad CTL responses by direct presentation in the absence of DCs (Bernhard et al., 2015). In many cases, immunodominant CD8 T cell responses against prevalent epitopes are sufficient to mount effective anti-pathogen effector and memory CTL responses. However, for pathogens or tumors with effective immune evasion, escape from most CD T cell responses occurs. Then it is of great advantage to have a broad CTL repertoire for protection. However, the factors determining the capacities of vaccines to induce broad CTL responses are incompletely understood. Side-by-side comparison of CTL responses initiated by different APC types and direct comparison of vaccine vectors such as rAd and modified vaccinia virus Ankara (MVA) revealed differential potencies to induce broad vs. narrow CTL responses.

Here, we analyse vaccine-intrinsic properties as well as their effects on lymphatic organs and cross-presenting XCR1+ DCs that may determine the strength, breadth and quality of CTL responses after vaccination.

P.A5.01.04
In vivo effect of environmental pollution on the expression of CD64, CD104, INFγR, CD206 and AHR receptors in human alveolar macrophages infected with Mycobacterium tuberculosis

C. Carranza, M. Perez-Guzman, M. Torres
National Institute of Respiratory Diseases, Mexico City, Mexico.

Introduction: The effects of air pollution on human health are a matter of great concern as more than two million deaths have been estimated to occur each year worldwide from direct damage to the respiratory system. Alveolar macrophages (AM) play an important role in the elimination of Mycobacterium tuberculosis (MTb) and are efficient in ingesting contaminating particulate matter (PM) that have penetrated the barriers of innate immunity. The load, composition and size of PM could determine the innate and adaptive immune responses of AM. Materials and Methods: We studied the in vivo effects of PM on human anti-mycobacterial host immunity in AM obtained from healthy subjects living in Mexico City. We also evaluated whether PM induces in vitro changes in AM (INFγR, CD104, CD206 and CD206membrane receptors, which are important in the protective response against MTb infection. At the same time we evaluate the induction of the arylhydrocarbon receptor (AhR), that senses environmental toxins like PM. Results: PM reduced CD64 expression and increased CD206 and AHR expression, which suggests that the protective response against MTb may be altered. The INFγR and CD104 receptors were not affected in response to PM. Conclusions: PM exposure affects immunity response against MTb in AM. AM activation may be altered by decreased expression of CD64 while the anti-inflamatory response is favored by the expression of CD206 and AhR.

P.A5.01.05
Intravaltral imaging of neutrophil dynamics after LPS administration

N. Chen, E. v. Grinsven, L. Koedermar, N. Vriskelop, Laboratory of Translational Immunology, Utrecht, Netherlands.

Upon acute inflammation evoked by intravenous infusion of lipopolysaccharide (LPS) in healthy human volunteers, neutrophil numbers in the blood initially decrease the first 90 min and subsequently dramatically increase. During this neutrophilia extra neutrophil subsets appear in the blood that can be distinguished based on nuclear morphology and expression of CD16 and CD62L. Banded CD16- as well as hypersegmented CD62L+ neutrophils appear in the blood.

In this study we aimed to establish the mechanism behind the neutrophilia found during the first 90 minutes after LPS administration. In addition we are investigating whether CD62L- neutrophils shed CD62L directly in the blood stream, ii) loose CD62L upon rolling along the blood vessel, or iii) whether CD62L+ neutrophils are recruited to the blood from another location.

Intravaltral microscopy has revolutionized biomedical research during the last two decades and has been used extensively to study dynamic process in intact tissues of living animals. Here we intravital image neutrophil dynamics and CD62L expression in blood vessels of the mouse ear upon LPS administration. During the first 90 minutes post LPS administration many neutrophils were found to decrease their speed and crawl along the blood vessels which could explain the lower neutrophil numbers collected in blood withdrawals. Some infrequent extravasation events could also be recorded. After 90 minutes more neutrophils started to appear in the blood stream, closely matching the dynamics found in blood withdrawals of both humans and mice post LPS administration. Upcoming experiments will image CD62L expression of neutrophils upon LPS administration.

P.A5.01.06
Propolis effects on human CD4+ T cells activation by LPS-treated dendritic cells

B. J. Condé, K. B. Santiago1, E. O. Cardoso1, F. L. Conti1, J. P. Oliveira1, K. L. Tasca1, M. A. Golim2, M. T. Cruz2, J. M. Sforcin1
1Department of Microbiology and Immunology, Biosciences Institute, UNESP, Botucatu, Brazil, 2Integrated Regional Faculties of Avař, Avař, Brazil, 3Laboratory of Flow Cytometry, Blood Center, School of Medicine, UNESP, Botucatu, Brazil, 4Center for Neurosciences and Cellular Biology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

Dendritic cells (DCs) represent a heterogeneous population of professional antigen presenting cells and are essential for recognition and presentation of pathogens to T cells. Propolis is a bee product demonstrating several biological properties, including its immunomodulatory effects. Here we intravital image neutrophil dynamics and CD62L expression in blood vessels of the mouse ear upon LPS administration. During the first 90 minutes post LPS administration many neutrophils were found to decrease their speed and crawl along the blood vessels which could explain the lower neutrophil numbers collected in blood withdrawals. Some infrequent extravasation events could also be recorded. After 90 minutes more neutrophils started to appear in the blood stream, closely matching the dynamics found in blood withdrawals of both humans and mice post LPS administration. Upcoming experiments will image CD62L expression of neutrophils upon LPS administration.

P.A5.01.07
The circadian clock protein Bmal1 regulates IL-1β in macrophages via NRF2

1Trinity biomedical sciences institute, Dublin, Ireland, 2Royal College of Surgeons, Dublin, Ireland.

The molecular clock, also termed the circadian clock, is the timekeeping system within all our cells that integrates many aspects of our biology to align with the 24 hr external environment. Cells of the innate immune system, including macrophages, have a robust molecular clock. BMAL1 is the major regulator of the molecular clock and its deletion affects macrophages in a pro-inflammatory phenotype. However the molecular mechanisms by which the molecular clock controls inflammation are not fully known. We have discovered that Bmal1 is crucial in triggering antioxidant defense in macrophages. Using a systems level approach, we demonstrate that macrophages lacking Bmal1 (and thus lacking a functional molecular clock) have diminished activity of NRF2, a key antioxidant transcription factor. Bmal1 knockout macrophages have reduced levels of NRF2 protein and lower induction of NRF2 target genes including Hmox-1 and Gsr following lipopolysaccharide (LPS) stimulation. The master antioxidant, glutathione, whose synthesis relies on NRF2 activity, is also severely reduced in cells lacking Bmal1.
Such impairments in NRFP2 mediated antioxidant defense result in increased production of reactive oxygen species (ROS) in BmM1/α-macrophages. Deletion of BmM2 boosts the transcription, translation and cleavage of the pyrogen IL-1β, a cytokine well documented to be under regulation by ROS and NRFP2. We have shown that use of antioxidants or enhancement of NRFP2 activity can rescue the pro-inflammatory phenotype of BmM1/α-macrophages. These findings uncover a novel mechanism by which the circadian cycle can control disease progression in inflammatory diseases such as sepsis, asthma and rheumatoid arthritis.

P.AS.01.08
Liver injury after acute CCL1 administration is independent of Smad7 expression in myeloid cells
J. Endrigi, D. Gotzl, P. Sprezyna, L. Diehl
Institute of Experimental Immunology and Hepatology, Hamburg, Germany, Institute of Pathology, Bonn, Germany.

Myeloid cells are essential for liver homeostasis, initiation and determination of innate and adaptive immunity. Smad7 is an inhibitor of the transforming growth factor-β signaling pathway that not only regulates T cell differentiation but also inflammatory cellular processes. Knockdown of Smad7 in hepatocytes has been shown to promote liver fibrosis, but little is known about the effects of Smad7 in myeloid cells during inflammatory responses in the liver. Using mice with a myeloid specific knock-down of Smad7 (LysM-Cre;Smad7−/−) we investigated the impact of Smad7 deficiency in myeloid cells on liver inflammation and regeneration using the well-established model of CCl4-mediated liver injury. Early (24/48h) and late (7d) time-points were analysed. We found that CCl4 induces a severe liver injury, with elevated serum ALT levels, centrilobular and periportal necrosis, infiltrating myeloid cells, and increase of inflammatory cytokines in the liver. Furthermore, as expected, inflammation peaked at 24h and subsided after 7d. However, there were no significant differences in the investigated parameters between the Smad7 wild type and Smad7−/− knock-down treatment groups. Thus our results suggest that inhibiting TGFβ via Smad7 expression in myeloid cells is dispensable for the induction and control of liver injury.

P.AS.01.09
The role of inflammasomes in human dendritic cell subsets
Department of Dermatology, University Hospital Erlangen-Nürnberg, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Erlangen, Germany, Department of Pathology, University Hospital Erlangen-Nürnberg, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Erlangen, Germany, Department of Chemistry and Pharmacy, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany, Department of Pediatric Cardiac Surgery, University Hospital Erlangen, Friedrich-Alexander- University Erlangen-Nürnberg (FAU), Erlangen, Germany, Institute of Neuropathology, Medical Center - University of Freiburg, Freiburg, Germany.

Dendritic Cells (DCs) are potent antigen presenting cells and required for connecting innate to adaptive immune responses. Therefore inflammasomes, important for the defense against, and for sensing many different ligands, play a crucial role in DC mediated immune responses. Growing evidence suggests an important function of inflammasomes in murine DCs but the specific role in the distinct human DC subsets has yet to be determined. In this ongoing study we show that stimulation of human CD1c+ DCs with inflammasome ligands after TLR engagement induces the secretion of TH1 and TH17 polarizing cytokines, potentially resulting in an enhanced T cell response in contrast to TLR stimulation alone. Stimulation of CD1c+ DCs with activators of NLRP1 or NLRP3 after TLR 7/8 engagement showed strong secretion of IL-1β, IL-12 and IL-23. Moreover, applying caspase 1 inhibitors effectively inhibited IL-1β secretion and therefore inflammasome and caspase activation. Activation of inflammasomes is frequently accompanied by pyroptosis, an inflammatory form of programmed cell death. Activating inflammasomes while maintaining cell viability was termed hyperactivation and has been described for OxPAPC, a mixture of oxidized phospholipids, in murine BMDCs. LDH release assays revealed only a slight or no cytotoxic effect for a few inflammasome activators in contrast to classical NLRP3 inflammasome activators such as ATP or Nigericin. Taken together, our results suggest promising effects for particular inflammasome ligands as potential hyperactivating adjuvants for boosting DC mediated immune responses.

P.AS.01.10
Activation of MIF(NF-κB) macrophages by Porphyromonas gulae LPS
Oral Health CRC, The University of Melbourne, Melbourne, Australia.

Porphyromonas gulae is an anaerobic Gram-negative bacterium that has been associated with periodontal disease in companion animals. Lipopolysaccharide (LPS) is a known virulence factor in bacteria such as Porphyromonas gingivalis, a closely related bacterium associated with chronic periodontitis in humans. However, the activity of P. gulae LPS has yet to be investigated. P. gulae LPS induced a similar level of IL12 activation compared to P. gingivalis LPS, both significantly impaired compared to LPS. P. gulae and P. gingivalis LPS also had TLR2 activity; which could be partially reduced with lipoprotein lipase. P. gulae and P. gingivalis LPS activated interferon-gamma treated macrophages as evidenced by CD86 expression and low levels of nitric oxide synthesis, which was dependent on TLR2 signalling and partially dependent on the TLR4 signalling pathway. P. gulae LPS induced higher levels of inflammatory cytokines than P. gingivalis LPS, comparable to that induced by E. coli LPS, which was completely sensitive to deletion of the TLR2 signalling ability. These results demonstrate P. gulae may have a modified lipid A moiety and thus avoids TLR4 activation, similar to P. gingivalis.

P.AS.01.11
Gene expression profiles of human resident lamina propria dendritic cells predict a functional role in tissue repair and angiogenesis under inflammatory conditions
Department of Immunology, Eötvös Loránd University, Budapest, Hungary, Klinikum Oldenburg, Oldenburg, Germany, University Hospital Heidelberg, Institute of Pathology, Heidelberg, Germany, University Hospital Heidelberg, Heidelberg, Germany.

Human intestinal lamina propria dendritic cells (LPDC) are known to be important regulators of intestinal adaptive immune responses. By extending dendrites through the intestinal epithelial layer they are capable of sampling intestinal luminal antigens. They subsequently transport these antigens to the mesenteric lymph nodes where they induce the recruitment of antigen specific T cells. In contrast to their impact on intestinal adaptive immune responses, local functions of lamina propria DCs during intestinal inflammation remain mostly unknown. Using a human intestinal organ culture model, we demonstrate that -under inflammatory conditions- CD45+ lineage, CD14+, CD11c+ myeloid LPDC are not only able to migrate out of the lamina propria onto the luminal side of the basement membrane following epithelial cell depletion. They also express higher levels of genes encoding inflammatory cytokines and chemokines such as IL-6, IL-1β, and CCL22 as well as of mediators associated with tissue repair (IMMP12, CTG5, BMP6) and angiogenesis (VEGFA) when compared to PB-DC. Interestingly, we observed increased mRNA levels of the IL-12 family cytokines subunits IL23a and IL12b while the mRNA levels of the corresponding binding partners IL12/p40 (for IL23p19), IL12-p35 and IL27-p28 (for EBI3) were not significantly increased. IL23p19 and EBI3 could potentially form the newly discovered IL-12 family member IL-39. According to recent studies IL-39 is involved in the regulation of inflammation by promoting neutrophil differentiation/expansion as well as wound healing. These results implicate that human resident LP-DC are not only regulating innate and adaptive immune responses but have a much broader spectrum of tasks.

P.AS.01.12
The role of CR3 (CD11b/CD18) and CR4 (CD11c/CD18) in adherence under physiological and inflammatory conditions
S. Lukácsi, T. Greerez, B. Franze, B. Saxel, R. Horváth, A. Erdő, Z. Bajaji
Department of Immunology, Eötvös Loránd University, Budapest, Hungary, MTA-ELTE Immunology Research Group, Eötvös Loránd University, Budapest, Hungary, Department of Biological Physics, Eötvös University, Budapest, Hungary, Nanobiosensors “Lendület” Group, Institute of Technical Physics and Material Sciences, Centre for Energy Research, Hungarian Academy of Sciences, Budapest, Hungary.

CR3 and CR4 belong to the family of β2-integrins and play an important role in phagocytosis, cellular adherence and migration. CR3 and CR4 are generally expected to mediate similar functions due to their structural homology and overlapping ligand specificity. Previously we proved that CR4 is dominant in the adhesion of monocytes, monocyte derived macrophages (MMDs) and monocyte derived dendritic cells (MDDCs) to fibrinogen under physiological conditions. Here we studied the expression of CR3 and CR4 and their participation in adherence to fibrinogen in inflammatory conditions induced by LPS. The expression of CR3 and CR4 was monitored by flow cytometry at different time points during the LPS induced cell activation. Cell adhesion to fibrinogen was evaluated using a state-of-the-art biophysical method, namely the force of cell attachment was measured with a computer controlled microfluidic system using vacuum assisted fluid flow. Comparing the amount of CR3 and CR4 on the cell surface we found that LPS treatment alters their expression differently on MMDs and MDDCs. Whereas on MMDs the expression of both CR3 and CR4 decreases (44% and 64%, respectively compared to non-treated control cells), on MDDCs CR4 shows a significantly elevated level (up to 147% compared to non-treated control cells), while that was only moderately improved in MDDCs. Using blocking antibodies we proved that adherence to fibrinogen is dominated by CD11b in both physiological and inflammatory conditions.
POSTER PRESENTATIONS

P.AS.01.12

Neutrophil-derived extracellular vesicles promote Th17 cell development
S. Mafteiu1, E. W. Toaman-Kuetter1, M. H. Woubere2, E. C. De Jong1, T. Groot Kormelink1

1Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, 2Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.

Th17 cells are important effector cells in fighting extracellular pathogens and are crucial players in the pathology of autoimmune disorders. We have recently demonstrated that neutrophil-derived elastase is essential for DC-driven Th17 cell development. However, it is yet unknown whether the uptake of elastase by DCs is important, and if extracellular vesicles (EVs) are involved in this process. In this study, we investigated the role of neutrophil-derived EVs in the transfer of elastase and the induction of Th17 cells. Neutrophils were isolated from blood of healthy donors and cultured in the absence or presence of activating stimuli: TNFα, LPS, fMLP. After stimulation, cells were collected and survival and activation were analyzed using flow cytometry. EVs were isolated from supernatants by differential centrifugation. For quantification by high-resolution flow cytometry, this step was followed by density ultracentrifugation. We found that neutrophil activation by TNFα and LPS did not enhance EV release compared to non-activated neutrophils. Surprisingly, fMLP activation resulted in the release of less EVs. Next we evaluated the capacity of neutrophil-derived EVs (EV-enriched 100,000xg pellets) to induce Th17 cell development from naïve CD4+ T cells in co-cultures with A. Albicans-activated monocyte-derived dendritic cells. Preliminary data suggests that EVs derived from TNFα/LPS-activated neutrophils, but not EVs from non-activated and fMLP-activated neutrophils, enhance Th17 cell development. These results indicate that activated neutrophils release EVs that promote DC-driven Th17 cell development. Our findings highlight a yet undescribed mechanism on the control of adaptive immunity by innate immune cell-derived EVs.

P.AS.01.15

LPS-induced neutrophil activation is modulated by new anti-PDE4 compounds
A. Moniot1, C. Guillaume1, I. Allart-Simort1, J. Sapir2, S. Gérad1, S. C. Gangloff1, P. Velard1

1UMR CNRS 7132 ICIM, Reims, France.

Polymorphonuclear Neutrophils (PMNs) are inflammatory cells, whose activation leads to abundant secretion of interleukin-8 (IL-8) and matrix metalloprotease 9 (MMP-9). To limit exacerbated cells response and tissue damages, anti-inflammatory molecules are commonly used. In this field, novel therapeutics are constantly sought to increase the anti-inflammatory arsenal. Type IV phosphodiesterase (PDE-IV) is able to hydrolyze cyclic adenosine monophosphate (cAMP) into AMP. PDE-IV related activity is increased in inflammatory processes. In this context, the PDE-IV inhibitors have been particularly promising.

In the here-presented in vitro work, we have tested five pyridazinone scaffold-based PDE-IV inhibitors for their capability to modulate intracellular cAMP and the expression of IL-8, MMP-9 and TNF-α by human neutrophils. Our results confirmed that pro-inflammatory stimulus (LPS) induced a decrease in intracellular cAMP level, this decrease has been counteracted by all our molecules. None of them impaired neither mRNA expression (RT-qPCR) or protein secretion (ELISA, zymography) in basal condition. In pro-inflammatory condition, IL-8, TNF-α and MMP-9 concentration was increased in neutrophils stimulated with LPS (three-fold, thirty-fold and two-fold respectively, p<0.05) whereas no variation has been seen at the mRNA level. Of interest, zardaverine (positive control for PDE-IV activity inhibition) and our PDE-IV inhibitors were able to decrease IL-8 and TNF-α secretion by at least 20% and 65% versus LPS condition respectively, as well as MMP-9 activity by 33%.

Taking together, our data show that pyridazinone derivatives may be interesting candidates as therapeutics against inflammatory diseases as they demonstrated anti-PDE-IV activity and seemed to be able to limit neutrophil activation.

P.AS.01.16

HLA-DR, CD4 and CD45-ROI in neutrophil extracellular traps
F. M. Rodriguez, I. Novak

Institute of Cell Biology, Faculty of Medicine, Cordoba, Argentina.

Introduction: Neutrophil polymorphonuclear leukocytes (PMN) may express costimulatory B7 molecules: CD80 and CD86 and molecules of MHC II: HLA-DR after stimulation with pro-inflammatory stimulus. Neutrophils are inflammatory cells, whose activation leads to abundant secretion of interleukin-8 (IL-8) and matrix metalloprotease 9 (MMP-9). In the here-presented in vitro work, we have tested five pyridazinone scaffold-based PDE-IV inhibitors for their capability to modulate intracellular cAMP and the expression of IL-8, MMP-9 and TNF-α by human neutrophils. Our results confirmed that pro-inflammatory stimulus (LPS) induced a decrease in intracellular cAMP level, this decrease has been counteracted by all our molecules. None of them impaired neither mRNA expression (RT-qPCR) or protein secretion (ELISA, zymography) in basal condition. In pro-inflammatory condition, IL-8, TNF-α and MMP-9 concentration was increased in neutrophils stimulated with LPS (three-fold, thirty-fold and two-fold respectively, p<0.05) whereas no variation has been seen at the mRNA level. Of interest, zardaverine (positive control for PDE-IV activity inhibition) and our PDE-IV inhibitors were able to decrease IL-8 and TNF-α secretion by at least 20% and 65% versus LPS condition respectively, as well as MMP-9 activity by 33%.

Taking together, our data show that pyridazinone derivatives may be interesting candidates as therapeutics against inflammatory diseases as they demonstrated anti-PDE-IV activity and seemed to be able to limit neutrophil activation.

P.AS.01.17

Differential imprinting of CD4+ T cell lung-homing capacity by conventional dendritic cells from inguinal and mediastinal lymph nodes
D. PEISSK1,2, P. Fontannaz1,2, S. Grillet1,2, D. Christensen4, P. Andersen2, P. Lambert1,2, C. Siegrist1,2

1Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland, 2World Health Organization Collaborating Center for Vaccine Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland, 3Center for Vaccinology, Geneva University Hospitals, Geneva, Switzerland, 4Statens Serum Institut, Copenhagen, Denmark.

Novel adjudants that instruct protective T cells to home to desired anatomical sites could be used to improve vaccine efficacy, for example against respiratory pathogens such as Mycobacterium tuberculosis. Dendritic cells (DCs) that drain different anatomical sites are capable of priming tissue-specific 'imprinting' signals during T cell priming, which favor the establishment of T cell populations that home to the original site of DC-antigen (Ag) encounter. The imprinting signals provided by DCs have been defined for several tissues though it is unclear whether a similar imprinting phenomenon occurs for lung trafficking T cells. We therefore used a Polyc/I (adjvant) and protein Ag vaccine model to compare airway and muscle-draining DCs in terms of phenotype and their ability to imprint T cell lung-homing markers. Our results demonstrate that Ag+ CD11b+ and CD103+ conventional DC subtypes are found in both intramuscular (LM) and intraluminal (IL) IL-17 T cell immunization. Importantly, cell-sorted Ag+ CD11b+ DCs from the MLN were superior, on a per-cell basis, to ILN-derived DCs at in vitro priming of CD4+ T cells with a lung-homing phenotype. The MLN-DC primed T cells then showed an enhanced capacity for in vivo trafficking to the lung parenchyma when transferred into naive mice, demonstrating that site-specific DC imprinting of lung-homing T cells occurs in this immunization model. Grant declaration: This work was supported by a Horizon2020 fund for TBVAC2020 project PHC-08-2014, paid by the Swiss Confederation SERFIR Contract 15.0038-4, 643381.
P.A.S.01.18
Glycan profiles of human macrophages and dendritic cells

E. Rapoport, E. Moiseeva, S. Khabdulakov, D. Aronov, G. Pazynina, S. Tsygankova, N. Bovin;
Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation.

Modification of vaccine particles with glycans promotes binding to dendritic cells (DCs) resulted in augmenting of vaccine efficacy. Therefore, we used a library of 229 fluorescent glycopyropes (Glyc-PAA-fluo) to select potential glycan “vector”. We found that the highest percent of probe-positive CD14+CD16-CD83+ subpopulation containing blood circulating DCs was observed for GaNAcα1-2Galb1-4(A2,3,6)-Man1-3(Man1-3GaNAcβ1-4)GalNAcβ1-4 and for three manose-reach glycans, namely (Ga1b1-4GalNAcβ1-3)Man1-3(Man1-3GaNAcβ1-4)GalNAcβ1-4 and (Ga1b1-4GalNAcβ1-3)Man1-3(Man1-3GaNAcβ1-4)GalNAcβ1-4. Mouse models are widely used to characterize different DCs subpopulations in vivo and in vitro as comparative genomics revealed functional differences between distinct human and mouse DC subsets. The aim of this work was to compare glycan-binding profiles of circulating in human and murine blood DCs. Murine circulating DCs were identified as CD11c+CD45-CD160+ in the blood of BY8R male mice. High percent of positive murine blood CD14+CD16-DC160+ cells were observed for A2,3,6-GaNAcα1-2Galb1-4(A2,3,6)-Man1-3(Man1-3GaNAcβ1-4)GalNAcβ1-4 and the other probes selected for human CD14+CD16-CD83+ cells influenced the T-cell response, which can contribute to the development of more efficient IAV vaccines against seasonal and pandemic IAV.

P.A.S.01.19
DOCK8 regulates macrophage migration through Cdc42 activation and LRAP35a interaction

T. Sakurai, A. Shiraishi, Y. Fukui;
1Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan, 2Research Center for Advanced Immunology, Kyushu University, Fukuoka, Japan.

Introduction: DOCK8 is an atypical guanine-nucleotide exchange factor for Cdc42, and its mutations cause combined immunodeficiency in humans. Accumulating evidence indicates that DOCK8 regulates the migration and activation of various immune cells, but its regulatory mechanism is not completely understood. Method and materials: Bone marrow-derived macrophages from DOCK8-/- or DOCK8+/+ mouse were used for chemotaxis assay, immunofluorescence staining, and biochemical analyses. Protein binding was assessed by immunoprecipitation, pull-down, and immunoblotting. Results: DOCK8-deficient macrophages exhibited a migration defect in a 2D setting. Although DOCK8 deficiency did not affect the global Cdc42 activity in macrophages, rescue experiments revealed that the guanine-nucleotide exchange factor activity of DOCK8 was required for macrophage migration. We previously reported that Cdc42 is regulated by LRAP35a in macrophages, and that LRAP35a interacts with Cdc42-binding protein 2 (Cdc2-binding protein 2) and facilitates its activity to phosphorylate myosin II regulatory light chain (MLC2). When this interaction was disrupted in wild-type macrophages, they showed a migration defect, as seen in DOCK8-deficient macrophages. Conclusion: DOCK8 links Cdc42 activation to actomyosin dynamics through the association with LRAP35a during macrophage migration.

P.A.S.01.20
Effects of propolis on human CD4+ T cells proliferation induced by MAGE-1-treated dendritic cells

K. B. Santiago1, 2, J. I. Conté, E. O. Cardoso, L. P. Oliveira, F. L. Conté, X. I. Tasač, M. A. Gailim, M. T. Cruz, J. M. Sfroncic;
1Department of Microbiology and Immunology, Biosciences Institute, UNESP, Botucatu, Brazil, 2Integrated Regional Faculties of Avaré, Avaré, Brazil, 3Laboratory of Flow Cytometry, Blood Center, School of Medicine, UNESP, Botucatu, Brazil.

The T-cell response in ferrets against influenza A virus is influenced by the site of infection, which IAV proteins are more likely to evoke an immune response and which proteins are involved in cross-protection. In a recent study, we infected ferrets (n=28) intranasal (i.n.) or intratracheal (i.t.) with H2N2 or PBS and analyzed samples from pre-infection and 14 days post infection. We found that i.n. infection with H2N2 invoked a stronger virus-specific T-cell response in the blood. T-cells showed strong responses against peptides of the conserved H2N2 H2N2 invoked a stronger virus-specific T-cell response in the blood.

P.A.S.01.21
Endocytosis of particulate matter of neutrophils induced oxidative stress through dynamin

y. yoshida, T. Miyake, D. Wang, M. Shen, K. Morita;

We previously reported the biological effects of PM in vivo, however, few reports have focused on the relationship between PM inhalation and neutrophils. Here, we investigated the effect of PM particle size on neutrophils. Flow cytometry analysis indicated that 1 μm particles are readily endocytosed by neutrophils and that endocytosis is reduced at 4°C. We previously reported the biological effects of PM in vivo, however, few reports have focused on the relationship between PM inhalation and neutrophils. Neutrophil-mediated endocytosis caused oxidative stress, and N-acetylcysteine enhanced endocytosis. Expression levels of the oxidative stress markers, heme oxygenase-1 and p62

P.A.S.02.01
Resolution of inflammation is altered in multiple sclerosis

V. Chirucli1, A. Leufl2, P. Norris, I. Riley, M. Albanese1, L. Battiaini, C. Serhan3;
1Neurochemistry of Lipids, European Center for Brain Research, Santa Lucia Foundation, Rome, Italy, 2Department of Medicine, Campus Bio-Medico University of Rome, Rome, Italy, 3Integrated Regional Faculties of Avaré, Avaré, Brazil, 4Neuroimmunology Unit, European Center for Brain Research, Santa Lucia Foundation, Rome, Italy.

Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease associated to uncontrolled inflammation and autoimmunity. Recent studies suggest that these can be a consequence of failure to resolve inflammation, a process that strictly depends on a newly discovered genus of highly potent anti-inflammatory lipids derived metabolically from omega-3 essential fatty acids and termed specialized pro-resolving lipid mediators (SPMs), that include resolvin, protectins and protexins. Here, by means of targeted metabololipidomics, we found that levels of specific SPMs such as lipoxins A4 and B4 as well as resolvin D1 (RvD1) and protectins P1 and PDX were increased in MS patients during the relapse phase, with RvD1 and PDI being also reduced or undetectable in the progressive phase. Principal Component Analysis (PCA) revealed differences in the production of specific pro-inflammatory mediators and SPMs according to the disease phase. Variations in the plasma levels of SPMs also significantly correlated with clinical scores and were observed in the different steps of the disease and with clinical scores. Furthermore, SPMs production was positively correlated with clinical scores and with the level of specific pro-inflammatory mediators.

P.A.S.02.02
The T-cell response in ferrets against influenza A virus is influenced by the site of infection

K. van de Ven, H. van Dijken, F. de Heij, J. de Jonge;
Notional Institute of Public Health and the Environment, Bilthoven, Netherlands.

Influenza A virus (IAV) infects millions of people each year, resulting in respiratory disease with symptoms ranging from a mild cold to severe fatal viral pneumonia. Vaccines may protect against multiple IAV subtypes by targeting conserved intracellular epitopes of IAV. By using ferrets, we can assess how the T-cell response against IAV is influenced by the site of infection, which IAV proteins are more likely to evoke an immune response and which proteins are involved in cross-protection. In a recent study, we infected ferrets (n=28) intranasal (i.n.) or intratracheal (i.t.) with H2N2 or PBS and analyzed samples from pre-infection and 14 days post infection. We found that i.n. infection with H2N2 involved a stronger virus-specific T-cell response in the blood. However, more CD8+ T-cells could be detected in the bronchoalveolar lavage of i.t. infected ferrets. T-cells showed strong responses against peptides of the conserved H2N2 proteins PA, PB1 and PB2, which corresponds with our observation that T-cells of H2N2 infected animals cross-react to H1N1. These results imply that the site of vaccination influences the T-cell response, which can contribute to the development of more efficient IAV vaccines against seasonal and pandemic IAV.
POSTER PRESENTATIONS

P.A5.02.03
Complement deficiency attenuated multiple organ failure in zymosan induced septic shock
P. Gomova1, V. Gyarkoska1, L. Belensko-Todorova2, N. Ivanovska1; 1Institute of Microbiology, Sofia, Bulgaria, 2Medical Faculty, Sofia University, Sofia, Bulgaria.

Septic shock is a complex inflammatory disease associated with a high rate of mortality. It starts with an overwhelmed immune response to infectious agents or their products in which the activated macrophages, neutrophils and the complement system play important roles. Cytokines and inflammatory mediators produced and secreted at first hours can induce organ failure and damage. Shock was induced by intraperitoneal injection of 1 mg/kg body weight of zymosan in BALB/c mice. Functional complement activity was evaluated by C5a (C5a) injection. The peritoneal cells were analyzed by flow cytometry for expression of C5aR1 (CD183) on monocytes/ neutrophil markers and dendritic cells. Plasma samples were analyzed for glucose, alanine aminotransferase (ALT), aspartate aminotransferase (ASTA), and bilirubin. Livers were conserved in paraffin blocks for histopathological examination. We have observed that complement depletion inhibited zymosan-induced organ dysfunction via decrease of liver injury and changes of hepatic enzyme levels. These effects were also with contamination of spleen and liver enlargement, and by reduced number of pro-inflammatory cells in the peritoneal cavity. Lack of functional complement, as occurred in anti-C5 antibody-treated mice, caused organ damage. Similar results were obtained by infusion of 20% of a complement-depleted plasma into the peritoneal cavity. These findings presented last showed that the development of organ failure can be positively influenced by an inhibition of the functional complement activity. Acknowledgements: This work was supported by a Grant DM 03/4, 17.12.2016 National Fund for Scientific Research, Bulgaria

P.A5.02.04
Simulating thrombocyte transfusions to investigate the impact of the self HLA background in HLA antibody formation and the risk for platelet refractoriness
K. Geneugeljik, T. de Hoop, E. Borst, E. Spierings; Laboratory of Translational Immunology, UMC Utrecht, Utrecht, Netherlands.

Platelet refractoriness is a rare condition in which thrombocytopenic patients fail to achieve sufficient platelet counts after thrombocyte transfusion. This condition can partially be ascribed to alloimmunization towards HLA-A and -HLA-B expressed on platelets. However, only a portion of patients will develop HLA antibodies after multiple transfusions. Previous studies have shown that mismatched HLA-derived T-helper epitopes presented by HLA class II (PIRCHIE-II) play a role in HLA-antibody formation. The aim of the current study is to investigate whether the patients’ HLA background may impact the ability to develop HLA antibodies upon thrombocyte transfusion. To this end, a representative patient population was generated by extracting all HLA-A, -B, -C, -DRB1, -DQB1 HLA typings that were performed in our center between January 2009 and July 2016. A virtual Caucasian thrombocyte donor pool of 10 million individual donors was modeled using HLA haplotype frequency tables. This virtual donor population was used to simulate 20 thrombocyte transfusions. PIRCHIE-II numbers were calculated for each simulated thrombocyte transfusion. The maximal number of unique T-helper epitopes that patients encountered after 20 simulated thrombocyte transfusions ranged between 31 and 359 epitopes. All patients had encountered half of their maximal T-helper epitopes after only two thrombocyte transfusions. Our simulations show that the maximal number of unique T-helper epitopes after multiple thrombocyte transfusions is highly variable between patients, suggesting a potential role of the self HLA background in the ability to become platelet refractory.

P.A5.02.05
Anthralin-induced skin inflammation is promoted by mast cells
A. Hartmann1, J. Sahil1, J. Ringen2, V. Tsivilovsky2, M. Brost3, H. Schild3, M. Freichel1, T. Feyerabend4, H. Redweik5, M. Radaski6, M. Stassen7; 1Institute for Immunology, University Medical Center Mainz, Mainz, Germany, 23rd Medical Department of the University Medical Center, Mainz, Germany, 3Institute of Pharmacology, University of Heidelberg, Mainz, Germany, 4Department of Dermatology, University Medical Center, Mainz, Germany, 5Institute of Pharmacology, University of Heidelberg, Hamburg, Germany, 6German Cancer Research Center, Heidelberg, Germany.

Psoriasis is an inflammatory skin disease characterized by abnormal proliferation of keratinocytes triggered by the cytokines IL-17A and IL-22. Due to its anti-psoriatic and anti-inflammatory action, the natural anthraquinone derivate anthralin is used for the effective treatment of psoriatic plaques without causing severe side effects. Whereas the underlying mechanisms are still not known in detail, anthralin affects growth and proliferation of skin cells. Interestingly, topical treatment with anthralin first enhances skin inflammation before psoriatic plaques begin to heal. In this context, increased mast cell numbers have been shown to be present in psoriatic plaques. Moreover, evidence is accumulating that mast cells contribute to the pathogenesis of psoriasis by secreting IL-17A and IL-22. Focusing the role of mast cells in anthralin-treated skin, we investigated whether mast cells are activated by anthralin in vitro and in vivo. In vitro, anthralin increases the concentration of intracellular Ca2+ in mast cells. Consequently, degranulation of mast cells and production of IL-6 are enhanced in the presence of anthralin. In vivo, anthralin causes severe skin inflammation, characterized by enhanced ear swelling, which is delayed in mast-cell-deficient Cpa3-Cre mice. Histological staining of murine ear skin showed an enhanced proliferation of epidermal cells and massive mast cell degranulation rates following topical application of anthralin. Taken together, these findings suggest that mast cells contribute to the inflammatory action upon topical anthralin treatment.

P.A5.02.06
Influenza A virus infection during pregnancy: elevated levels of pregnancy hormones alter immune reaction against influenza A virus
A. M. Hierweger1, G. Engels2, K. Thiele3, G. Gabriel1, H. Müttträcker1, P. C. Arcà2; 1Institute for Immunology, Hamburg, Germany, 2Department of Obstetrics and Fetal Medicine, Laboratory for Experimental Feto-Maternal Medicine, Hamburg, Germany, 3Viral Zoonooses and Adaptation, Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany.

During the 2009 H1N1 influenza A (IAV) virus pandemic, pregnant women showed higher influenza related morbidity and mortality. Thus, it is of great clinical interest to investigate the pathogenesis and underlying altered immune responses leading to increased risk for pregnant women. We previously developed an IAV mouse model where semiallogeneically, Balb/c-mated C57Bl/6j dams are infected with a 2009 pandemic H1N1 IAV strain. Pregnant mice demonstrate increased mortality and morbidity, which corresponds to the clinical observations in humans. Using this model, we showed reduced immune responses in pregnant IAV infected mice compared to non-pregnant littermates. Elevated progesterone levels during pregnancy lead to immune adaptation and could probably account for some of our observations in pregnant IAV infected mice. Therefore, we infected pregnant mice lacking the progesterone receptor in DCs and observed increased survival compared to pregnant infected mice of the control strain. As progesterone can signal via the progesterone and glucocorticoid receptor, mice lacking the glucocorticoid receptor on DCs and T cells will be analysed upon infection. Using this mouse model, we could show reduced dendritic cell (DC) activation and subsequent reduced CD8 T cell responses in pregnant IAV infected mice compared to non-pregnant IAV infected mice. These observations strongly support the concept that hormonal changes during pregnancy are relevantly involved in modulation of the immune response against the influenza virus. Whilst the immune modulation during pregnancy is advantageous for pregnancy maintenance it is associated with significant disadvantages for maternal health, mirrored by the increased risk for severe IAV infection.

P.A5.02.07
Cigarette smoke differentially affects inflammatory response depending on exposure time points in a mouse model of nonalcoholic steatohepatitis
J. Kim1, Z. Zhou2, H. Jeong2, S. Choi1, S. Lee3, W. Kim1, K. Lee1, B. Kim1; 1Biostatistics Research Institute and College of Veterinary Medicine, Chungbuk National University, Iksan, Korea, Republic of; 2Inhalation Toxicology Center, Jeonbuk Department of Inhalation Research, Korea Institute of Toxicology, Jeongeup, Korea, Republic of; 3Division of Biotechnology, College of Environmental and Biosource Science, Chungbuk National University, Iksan, Korea, Republic of.

Introduction: We tried to demonstrate the impact on main stream cigarette smoke (MSCS) to nonalcoholic steatohepatitis (NASH) progression in sexually mature mice. Materials and Methods: Mice were either fed a control diet or a diet containing a-methine-choline deficient with high fat diet for 6 weeks. During the first (early exposure) or last (late exposure) three weeks of diet feeding, each diet group was exposed to MSACS (300 or 600 μg/mL C5300 or C5600) for 2 hours per day and 5 days per week. CS extract (CSEx) was extracted from 3RF reference cigarettes and used for ex vivo study. Results: Hepatic or serum biochemical analysis showed that MSACS differentially modulated hepatic injury in NASH mice depending on exposure time points. Consistently, histopathologic observation provided that NASH severity was increased in early exposure group, but decreased in late exposure group except steatosis. Similar results were observed in the main hepatic markers and were confirmed by TUNEL assay and Sirius hepatoacellular spectvscopy. Our ex vivo experiments found that MSACS treatment differentially regulated inflammatory responses in co-cultured hepatocytes and macrophages isolated from liver with steatohepatitis after 10 days or 3 weeks of diet feeding. Furthermore, MSACS treatment differentially up- or down-regulated the expression levels of peroxisome proliferator-activated receptor-gamma (PPARγ) in co-cultured macrophages. Finally, CSE treatment differentially affects M1/M2 polarization in co-cultured macrophages. Conclusions: Our findings indicate that opposite effects of MSACS on NASH progression are mediated by differential modulation of PPARγ and its-associated M1/M2 polarization in hepatic macrophages depending on exposure time points.
Humanized mice harbouring cellular and molecular components of the human immune system (HIS) represent a ground-breaking preclinical platform to study human immune cell biology. Before evaluating novel immunotherapies in the context of chronic HIV infection, we aimed to evaluate the exhaustion and senescence profile of human T cells in blood and lymphoid tissues of HIS mice. In Balb/c Rag2−/−Rag1−/−Il2rg−/− mice reconstituted with human cord blood CD34+ cells, human hematopoietic cells represented 83% +/- 3.3% in spleen and 68% +/- 15.4% in bone marrow. The human T cell proportion as well as the HCA4: HCD8 T cell ratio were similar to those observed in humans. Naïve T cells represented the major T cell compartment in blood and blood whereas memory T cells were predominant in the bone marrow, as observed in humans. We next studied the basal expression level of exhaustion markers (such as PD-1 and TIGIT, that are important immune checkpoints) and senescence markers (CD57 and KLRG1). Human T cells developing in BrGF5-A2 HIS mice presented an exhaustion level similar to humans, while PD-1 expression being nearly constitutive in the bone marrow, whereas senescence level (as assessed by CD57) was slightly lower. Overall, the proportion, differentiation, exhaustion and senescence profiles of human T cells in BrGF5-A2 HIS mice resemble those observed in human studies. The physiological expression levels of immune checkpoints in humanized mouse models will allow us exploring anti-immune checkpoints strategies in HIV-1 chronic infection, in order to optimize the current antiretroviral treatment.

Our findings indicate that IL-17F polymorphism, rs763780, might be associated with a high risk of RPL in Iranian women.

Methods: In a case-control study performed on two groups consisting of 85 healthy women with at least one delivery and 85 women with the history of two or more RPLs. The frequency of IL-17A rs7275913 and IL-17F rs763780 polymorphisms were determined by PCR-RFLP.

Results: The genotype frequencies of rs7275913 polymorphism were GG (83.6 %), GA (61.2 %) and in the control group, were GG (3.5 %), AG (42.4 %) and AA (54.1 %). Statistical analysis showed no significant difference between the genotypes of AA, AG and GG in the two groups (p=0.1). The genotypes frequencies of rs763780 polymorphism were TT (43.5 %), TC (49.4 %) and CC (7.1 %) in the RPL group; whereas the frequencies were TT (25.9 %), TC (70.6 %) and CC (3.5 %) in the control group. Statistical analysis revealed a significant difference in the CC genotype between the case and the control groups (p=0.01).

Conclusion: Our findings indicate that IL-17F polymorphism, rs763780, might be associated with a high risk of RPL in Iranian women.
BBSome is a transport protein complex, which is important for normal formation and functioning of primary cilia. Mutations in BBSome subunits cause a severe multigorgan disease called Bardet-Biedl Syndrome (BBS). Interestingly, some of the proteins, which enable ciliary transport, also play a role in the formation of immunological synapse (IS), a contact site between an antigen-presenting-cell and a lymphocyte. However, possible impact of BBSome in this process has not been investigated yet. Using YFP-labelled BB54 subunit, we indicate that BB54 actually localize to the IS during its formation. In order to investigate possible influence of BBSome proteins on the immunity, we established mouse model of BBS (BB54KO). Comparison of WT and BB54KO mice confirm expression of BB54 in normal T- and B-cells. BB54KO mice have alterations in hematopoietic system, such as increased monocyte number, increased hemoglobin and elevated platelets number. Moreover, our preliminary data indicate that BB54KO mice have a partial impairment in B-cells development. Further research will shed light on the functions of BBSome proteins in immune cells.

[References]

N. J. Valeta, L. J. Uniorf, M. S. Ventimiglia, M. C. Abbou, M. F. Quiroga, F. Jensen;
1CEPFR-CONICET, Buenos Aires, Argentina, 2CINIBA-UNLP, Buenos Aires, Argentina.

**P.A5.02.14**

**IL-33 receptor (ST2) expression on B cells during pregnancy and preterm birth**

1Moredun Research Institute, Penicuik, United Kingdom, 2University of Edinburgh, Edinburgh, United Kingdom, 3Univesita degli Studi di Pergua, Pergua, Italy, 4Universidade de Leon, Leon, Spain.

**Introduction:** The desired goal of vaccines to prevent intoacellular bacterial infections is usually the induction of cellular T-helper (Th)-1 type immunity, characterised by the production of interferon-IFN)-gamma. This has been particularly challenging for subunit vaccine development in livestock. We have characterised cellular immune responses in sheep to an experimental chlamydial subunit vaccine antigen delivered in three adjuvants.

**Materials and Methods:** Groups of 35 sheep were immunised with a single inoculation of the experimental vaccine antigen formulated in two water-in-oil adjuvants (Montanide ISA 70 VG, Montanide ISA 61 VG) or saponin-derived QuilA. Peripheral blood mononuclear cells (PBMC) were isolated pre- and post-immunisation and re-stimulated in vitro with both the vaccine antigen and whole killed chlamydial elementary bodies (EBs). Recall responses were measured by the presence of cytokines in the PBMC culture supernatants, with IFN-gamma as an indicator of Th1-type responses and interleukin (IL)-4 as an indicator of Th2-type responses.

**Results:** All three adjuvants induced antigen-specific immune responses that could be detected in recall assays to both the experimental vaccine antigen and whole chlamydial EBs. Th1-type responses were observed throughout the experiment. Viral infection also induced a numerically stable CX3CR1int Tmem cells homed to lymph nodes, but CX3CR1high Tmem cells, predominantly surveyed peripheral tissues. As CX3CR1int Tmem cells present unique phenotypic, homeostatic, and migratory properties, we designate this subset peripheral memory (Tpm) cells.

**Conclusions:** Classical Th1-type responses can be elicited in sheep to a subunit antigen delivered in different adjuvants. The relative efficacy of these adjuvants needs to be determined using infection challenge models.

[References]

F. Ilića, D. Papatsenko, S. Andrianov; 2Harvard Medical School, Boston, United States, 3Kurilinska Institutet, Stockholm, Sweden, 4Harvard Medical School, Boston, United States.

**P.A5.02.15**

**Effect of adjuvant on immune responses to an experimental subunit vaccine antigen in sheep**

1Moredun Research Institute, Penicuik, United Kingdom, 2University of Edinburgh, Edinburgh, United Kingdom, 3Universita degli Studi di Pergua, Pergua, Italy, 4Universidade de Leon, Leon, Spain.

**Introduction:** The desired goal of vaccines to prevent intoacellular bacterial infections is usually the induction of cellular T-helper (Th)-1 type immunity, characterised by the production of interferon-IFN)-gamma. This has been particularly challenging for subunit vaccine development in livestock. We have characterised cellular immune responses in sheep to an experimental chlamydial subunit vaccine antigen delivered in three adjuvants.

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**Conclusions:** Classical Th1-type responses can be elicited in sheep to a subunit antigen delivered in different adjuvants. The relative efficacy of these adjuvants needs to be determined using infection challenge models.

[References]

F. Ilića, D. Papatsenko, S. Andrianov; 2Harvard Medical School, Boston, United States, 3Kurilinska Institutet, Stockholm, Sweden, 4Harvard Medical School, Boston, United States.
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POSTER PRESENTATIONS

P.AS.02.18
TLR2-mediated inflammatory responses and their impact on the course of dengue virus infection


1Department of Medical Microbiology, University of Groningen and University Medical Center Groningen, Groningen, Netherlands, 2Instituut Pasteur du Cambodge, International Network of Pasteur Institutes, Phnom Penh, Cambodia, 3Departments of Pathology & Medical Biology and Critical Care, University of Groningen and University Medical Center Groningen, Groningen, Netherlands.

Clinical manifestations of dengue virus (DENV) infections range from a flu-like to a severe disease hallmarking increased vascular permeability and/or plasma leakage. Excessive inflammation precedes severe disease; however, its underlying mechanisms are only partially understood. Consequently, there are no methods to predict or block DENV pathogenesis. Toll-like receptors (TLR) play a crucial role in the initiation of inflammation and containment of infections. Yet, prolonged activation of TLRs exacerbates inflammation, which ultimately leads to vascular immunopathology. We and others have shown increased expression of TLR2 on monocytes of DENV infected patients compared to healthy controls during the acute phase of infection. Here, we combined ex vivo and in vitro analysis to identify the relevance of TLR2 expression on peripheral blood mononuclear cells in DENV pathogenesis. We found that TLR2 expression on CD14++CD16 classical monocytes isolated during acute DENV infection correlated with disease severity in vitro, blocking of TLR2 prior to DENV infection abolished inflammatory responses mediated by NF-kappaB resulting in diminished intracellular cytokine production and attenuated human endothelial cell activation. Furthermore, blocking the engagement of TLR2 and CD14 but not that of TLR1/6 significantly reduced infected cell-mass, suggesting that DENV usurps TLR2 and CD14 to establish infection. Consistent with these findings, in patients, DENV infection was evident primarily in CD14++CD16 monocytes, which correlated with the development of severe disease. Conclusively, our data reveal the fundamental role of TLR2 as a regulator DENV-induced inflammation and immunopathology. Pharmacological targeting of the TLR2 axis could form a strategy for mitigating the pathogenesis of severe disease.

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P.AS.03.01
HIV-1 hijacks the complement system to escape degradation and promote viral dissemination by human Langerhans cells

M. Bermejo Lambrina, B. Nijnheer, D. Wilfingseder, T. Geijtenbeek;

1Department of Hygiene and Medical microbiology, Medical University of Innsbruck, Innsbruck, Austria, 2Innsbruck, Austria, 3Department of Experimental Immunology, Amsterdam Infection and Immunity Institute, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.

The role of complement in HIV-1 susceptibility remains unclear. In vivo, HIV-1 spontaneously activates complement in semen, plasma and mucosal surfaces and is therefore coated after viral entry. However, whether this is involved in viral transmission is unknown. Langerhans cells (LCs) reside in mucosal tissue and are the first cells that encounter HIV-1 during sexual contact. LCs are able to limit dissemination by degrading HIV-1 via langerin-induced autophagic processes, preventing HIV-1 infection of LCs and transmission to T cells. We set out to investigate the role of complement in sexual transmission of HIV-1 using isolated human LCs and the ex vivo skin transmission model. Strikingly, complement- osonized- HIV-1, in contrast to non-sonized HIV-1, efficiently infected LCs, in vitro and ex vivo. Moreover, complement-sonized-HIV-1 was efficiently transmitted to target T cells in the ex vivo skin transmission model. Infection and subsequent transmission of LCs were inhibited by blocking CR3 and CR4. Using isolated activated LCs we observed that complement osonization increased HIV-1 binding to LCs through CR3 and that LCs, Langerin inhibition reduced binding of complement- osonized-HIV-1 but both CR3 and CR4 were most important for binding. These data suggest that complement osonization leads to a different routing of HIV-1 in LCs via CR3 and CR4 binding, evading antiviral function of langerin and increasing HIV-1 infection of LCs and subsequent viral dissemination. This study provides novel insight into the importance of complement in HIV susceptibility and might lead to preventive strategies to prevent HIV-1 infection. This work was funded by EF5.

P.AS.03.02
Unraveling the regulation of Antigen-specific Immunoglobulin Glycosylation

E. L. de Graaf, P. Visser, A. Hijgrafe-Ederveen, C. Koeleman, E. van der Schoot, M. Wuhrer, G. Vidarsson;

1Sanquin Research, Amsterdam, Netherlands, 2Centre for Proteomics and Metabolomics, LUMC, Leiden, Netherlands.

We have previously shown that antibodies formed against epitope and red blood cell antigens can be skewed towards a unique type of N-linked IgG Fc-glycan profile with decreased fucosylation, increased galactosylation and sialylation. The lowered core-fucosylation increases the affinity of the pathogenic antibodies to FcγRIIIa and FcγRIIb, and hence platelet/ RBC destruction. More remarkably, the Fc-glyco profile seems to be stable for years and even decades after immunization. In order to understand how the IgG glycosylation is regulated on the B cell level, we have set up a B cell culture system where we can follow antigen-specific glycosylation in clonally related cells. By following the development of an early memory B cell (i.e. CD27+, IgM+) into a late state memory B cell (CD27+, IgG+) characterized by low and high somatic hypermutation, respectively, we attempt to determine the point of IgG glyco-memory formation. Firstly, antigen-specific B-cell clones from affected donors were isolated, FACS sorted and individually expanded in vitro. Thereafter, antibody glycosylation profiles were determined by measuring Fc glycopeptide abundances from each supernatant, using liquid chromatography and mass spectrometry. Subsequently, the acquired B-cell-specific IgG glycoprofiles will be correlated to the clonal relation and mRNA expression of a panel of glycosylating enzymes of each clone. Initial results will be presented, providing more insight in the regulation of antigen-specific antibody glycosylation.

P.AS.03.03
NK-cell mediated ADCC via FcγRlla is affected by IgG3 polymorphisms


Besides immunoglobulin isotypes and subclasses, polymorphisms in the immunoglobulin gamma heavy chain gene form another layer of variation to the humoral antibody response (IgG allotypes). From the four IgG subclasses (IgG1-4), most variation has been found in IgG3. Interestingly, several allotypes have been linked with susceptibility to various infectious diseases or auto-immune diseases. To study the influence of the polymorphisms on IgG effector functions, we produced all the described allotypes (27 allotypes, anti-RhD specificity) and subsequently assessed Fc gamma receptor (FcγR) binding with surface plasmon resonance (SPR). When we compared IgG3 allotypes, we observed small differences in FcγRlla binding. Most prominently, allotype IgG3*18 and IgG3*19 bound less well than FcγRlla than all the other IgG3 allotypes. The three-fold lower affinity of these allotypes for FcγRlla directly correlated with a reduced capacity to induce ADCC. Allotype IgG3*18 and IgG3*19 express a unique tryptophan at position 292 in the CH2 domain instead of an arginine, which is not found in the other IgG3 subclasses. Since residue 292 is not directly involved in binding to FcγRlla, we hypothesize that the variation at position 292 alters the conformation of the loop that interacts with FcγRlla, which is likely possibility based on available structural data. Future experiments are necessary to understand the interaction of IgG allotypes with FcγRlla, but also the neonatal Fc-receptor, and complement. These experiments should give new insights how these allotypes may possibly be linked with susceptibility to infectious diseases, allo- or auto-immune diseases.

P.AS.03.04
T cells enhance direct infection of NK cells by viruses, leading to enhanced effector functions

M. Jambregts, N. Swaans, M. Emmelot, E. A. van Erp, D. van Baarle, J. de Wit;

National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands.

Natural Killer (NK) cells are important in virus-infections and were originally believed to mainly recognize and eliminate infected cells through cytotoxicity. Increasing evidence now illuminates the immune modulatory functions of NK cells, via (pro-)inflammatory cytokines or intercellular interactions, underlining the complexity of NK-cell crosstalk with both innate and adaptive immune cells. Previously, we found a significant contribution of NK cell to the IFN-γ response against mumps virus (MuV). Here we sought to investigate the direct effects of virus-interactions with NK cells, and the crosstalk with other immune cells therein. Incubation with live MuV resulted in a substantial infection of isolated human NK cells (up to 45%). NK cell infection was even further enhanced in presence of other PBMCs (up to 80%), implying crosstalk with other immune cells. Indeed, depletion of T cells reduced the infection of NK cells. The T-cell mediated enhanced infection of NK cells was also observed for other virus species (including measles and respiratory syncytial virus), suggesting a broader phenomenon. Following infection, NK cells were strongly activated and displayed enhanced effector functions, as shown by IFN-γ production and release of granzymes and perforin. The implication of the enhanced effector functions on the NK-mediated immune modulation remains to be elucidated.
P.A5.03.05
Caspase 1 activity impairs CDB T-cells responses in coronavirus induced hepatitis
M. Duhalde Vega1,2, M. Jedrzej 1, M. Hill1,2
1Laboratory of Immunoregulation and Inflammation. Institut Pasteur de Montevideo., Montevideo, Uruguay, 2Institute of Biochemistry and Biophysics (IQUIFIB, UBA-CONICET), Buenos Aires, Argentina, 3Centro for Translational Immunology. FOCIS Centre of Excellence. Montevideo Faculty of Medicine, Institut Pasteur de Montevideo, Montevideo, Uruguay, 4Immunology Department. Faculty of Medicine. University of the Republic, Montevideo, Uruguay.

Introduction: The inflammases play a crucial role in the immune response to viral infection. Activation of inflammases triggers the cleavage of caspase-1 and maturation of IL1β. Monocytic cells act as an efficient infection control. The positive relation between IL1β release and adaptive immunity has been exhaustively described, but hepatitis seems to be negatively influenced by the inflammases. In HCV, IL1β level is augmented and it has been associated with immunopathology and viral load. Therefore, the aim of this work was to further analyze the role of the Caspase-1-IL1β axis in the initiation of adaptive immune responses. Methods: Cells and animals were infected with Mouse Hepatitis Virus strain A59 (MHV). We use WT and Caspase 1-deficient mice (Casp1−/−). Results: BMDMs derived from Casp1−/− and wt mice were infected with MHV and inflammases analysis was performed by flow cytometry. Data have showed that MHV infection induced IL1β release on WT mice, but not on Casp1−/− BMDCs. Then, in vivo studies have shown that Casp1−/− efficiency ameliorates MHV-induced hepatitis. Survival rates revealed that Casp1−/− mice are resistant to MHV infection, while only 25% of wt mice survive at 20dpi. In accordance, liver from Casp1−/− mice showed reduced levels of MHV-RNA and augmented liver CDB-Tcells infiltration. Moreover, Casp1−/− mice have elevated number of MHV-specific CDB-T-cells and higher expression of CD107a marker. Finally, in vivo CTL activity assay confirmed that Casp1−/− mice have higher MHV-specific CTL activity than wt mice. Conclusion: We found that Caspase 1 activity is crucial in the modulation of CDB-T-cell response to coronavirus-induced hepatitis.

P.A5.03.06
A phosphatidylinositol 4, 5-biphosphate (PIP2) metabolism-derived amplification loop fuels the sustained initiation of B cell activation
W. Liu, C. Xu
TSINGHUA UNIVERSITY, BEIJING, China.

Lymphocytes have evolved sophisticated signaling amplification mechanisms to efficiently activate downstream following detection of rare ligands in their microenvironment. B cell receptor microclusters (BCR microclusters) are assembled on the plasma membrane and recruit signaling molecules for the initiation of lymphocyte signaling after antigen binding. Here, we identified a signaling amplification loop derived from phosphatidylinositol 4, 5-biphosphate (PIP2) for the sustained B cell activation. Upon antigen recognition, PIP2 was depleted by phospholipase Cγ2 (PLCγ2) within the BCR microclusters and was regenerated by phosphatidic acid (PA)-dependent type 1 phosphatidylinositol 4-phosphate 5-kinate (PIP5K) outside of the BCR microclusters. The hydrolysis of PIP2 inside of the BCR microclusters induced a positive feedback mechanism for its synthesis outside of the BCR microclusters. The falling gradient of PIP2 across the boundary of BCR microclusters was important for the efficient formation of BCR microclusters. Our results identified a PIP2-derived amplification loop that fuels the sustained initiation of B cell activation.

P.A5.03.07
Anti-domain 1 beta2-glycoprotein I antibodies induce activation of monocytes and NK cells, and provoke prothrombotic settings
A. Martirosyan1, T. Papajik1, S. Margaronian1,2, Z. Nikulova1, L. Slavik1, J. Ulehlova1, E. Kriegova1, G. Manukyan1,2
1Laboratory of Molecular and Cellular Immunology, Institute of Molecular Biology NAS RA, Yerevan, Armenia, 2Department of Hemato-oncology, Faculty of Medicine and Dentistry, Palacky University Olomouc and Faculty Hospital, Olomouc, Czech Republic, 3Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc and Faculty Hospital, Olomouc, Czech Republic.

Introduction: It has been suggested that antibodies against domain I (D1) of beta2-glycoprotein I (β2GPI) have clinical relevance in antiphospholipid syndrome (APS) patients, and strongly correlate with thrombosis and pregnancy complications. The direct influence of anti-D1 β2GPI on activation and pro-thrombotic activity of immune cells has not been studied yet. We aimed to determine the influence of anti-D1 β2GPI IgG antibodies on immune cells in vitro. Methods: For this, peripheral blood mononuclear cells from 11 healthy individuals were incubated (for 24 hours) with: 1) pooled plasma (n=6) derived from APS patients contained anticardiolipin antibodies (aCL), lupus anticoagulant (LA), anti-β2GPI and anti-D1 β2GPI; 2) pooled plasma (n=5) derived from APS patients contained aCL, LA, anti-β2GPI, and negative for anti-D1 β2GPI; 3) seronegative (negative for antiphospholipid antibodies) pooled plasma (n=6). Results: The presence of anti-D1 β2GPI markedly induced a proinflammatory phenotype of monocytes and NK cells in comparison with the cells cultured with anti-D1 β2GPI-negative and seronegative plasma. Particularly, anti-D1 β2GPI significantly increased % of MFI of CD142 (tissue factor, TF), HLA-DR and CD11 on healthy monocytes. A greater percentage of CD69+ NK cells was found upon cultivation with anti-D1 β2GPI+ plasma. Expression of IgG receptor FcγRIIIa (CD16) on both monocytes and NK cells was down-regulated by anti-D1 β2GPI + plasma. Conclusion: Taking together, for the first time, we demonstrated strong activation of monocytes and NK cells exposed to anti-D1 β2GPI. Prominently, anti-D1 β2GPI induced substantial increase in expression of monocyteic TF favoring an initiation of thrombus formation. Grant support: IGA UP_2018_016

P.A5.03.08
Antigen-specific activation of murine B lymphocytes in vitro
S. Michelchen, H. Hanack
University of Potsdam, Department of Biochemistry and Biology, Potsdam, Germany.

Introduction: The generation of monoclonal antibodies by hybridoma technology is currently performed by immunizing animals such as mice with the desired antigen. The in vivo approach takes place in vivo without any opportunities to intervene. The transfer of antigen-specific immune responses to in vitro conditions would allow a monitoring of these processes in a defined culture environment. For this, a simplified set-up was established in which only B lymphocytes were cultured with supplements mimicking the in vivo conditions of antigen-specific activation.

Methods: Murine splenic B lymphocytes from naive mice were isolated by magnetic cell sorting and cultivated in vitro with different combinations of antigens, aCD40-antibody, LPS, IL4 and IL7. As antigen a viral protein from the hamster polyomavirus capsid (VP1) was used. Antibody responses were determined by ELISA and Western Blot. B cell phenotypes were investigated by flow cytometry. Positive cultures were used to generate stable antibody producing hybridomas.

Results: VP1-specific antibody responses in in vitro cultures could be detected from day 3 on with specific IgM-responses. At day 9 we detected specific IgG-antibodies in cultures stimulated with anti-D1 β2GPI+ plasma. Expression of IgG receptor FcγRIIA (CD16) on both monocytes and NK cells was down-regulated by anti-D1 β2GPI + plasma. Expression of IgG receptor FcγRIIIa (CD16) on both monocytes and NK cells was down-regulated by anti-D1 β2GPI + plasma. Conclusion: Taking together, for the first time, we demonstrated strong activation of monocytes and NK cells exposed to anti-D1 β2GPI. Prominently, anti-D1 β2GPI induced substantial increase in expression of monocyteic TF favoring an initiation of thrombus formation. Grant support: IGA UP_2018_016

P.A5.03.09
CD27-mediated stimulation of human CD4 T-cells leads to a reduction in active HIV-production
S. Nüssing1, R. M. van der Sluis1, T. H. Nguyen1, J. L. Anderson1,2, K. Kedzierska3,4
1Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia, 2School of Medical Sciences, UNSW, Sydney, Australia, 3Institute of Biochemistry and Biophysics (IQUIFIB, UBA-CONICET), Buenos Aires, Argentina, 4Centre for Translational Immunology. FOCIS Centre of Excellence. Montevideo Faculty of Medicine, Institut Pasteur de Montevideo, Montevideo, Uruguay.

Background: CD27 (CD70) is a co-stimulatory receptor that is crucial for the development and maintenance of adaptive immunity. CD27 engagement on T-cells leads to a reduction in active HIV-production

Methods: Firstly, we infected peripheral blood mononuclear cells (PBMCs) with the SHIV /89 rev strain. PBMCs were cultured for 14 days and IL-2 was added. CD27 stimulation was performed in the presence of IL-2. Data was analyzed by flow cytometry for the expression of CD40L, CD137, CD69, and CCR5. Results: Data indicated that CD27 stimulation decreases the expression of CD40L and CD137, while increasing the expression of CD69 and CCR5. Conclusion: CD27 stimulation decreases HIV-production, while increasing the expression of CCR5. Further studies are needed to determine the mechanism of CD27-mediated stimulation of human CD4 T-cells in HIV-production.
POSTER PRESENTATIONS

P.A5.03.10
Cell adaptation of monoclonal antibodies produced by Chinese hamster Ovary (CHO) cell to grown in serum-free medium
Wi. Puangmanee, Faculty of Tropical Medicine, Bangkok, Thailand.
Wilarat Puangmanee1, Pongrama Ramasoota1, Pannanthip Pitaksakijul
1 Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University
Monoclonal antibody (MAb) is one of the most widely used substance for biopharmaceuticals and therapeutics for viral disease, especially dengue virus which is one of important re-emerging virus causing life-threatening disease around the world. MAb can be produce using immunoglobulin G (IgG) gene cloning and expression in Mammalian Chinese Hamster Ovary (CHO) cell. Therefore, in vast value, it is necessary to produce in efficient large-scale production for control quality of MAbS. From our successful generation, human monoclonal antibodies (HuMAbs) were produced by fusion between myeloma cells and B-cells using hybridoma technology. These HuMAbs showed cross-neutralizing activity to dengue virus 4 serotypes. The recombinant IgG form of those HuMAbs showed prefer neutralizing (NT) activity.
For further characterization and standardization of HuMAbs, generation of stable CHO cells for HuMAb production is required. In this study, plasmid of HC and LC were transfected to CHO cell using lipofectamine 2000. Then, stable CHO cells were selected by two antibiotics, puromycin and hygromycin. Then, high producer CHO cell was selected by flow cytometer. The single clones were subcloned by limiting dilution. Single clone that showed high production of HuMAb were screened by IgG quantitation ELISA. Stability of stable CHO cell was tested. The clones that showed high stability in antibody production were re-cloned and proceeded for suspension cell adaptation and production in serum-free medium for further scale up.
Keywords: Human monoclonal antibody, Mammallian cell, Neutralization activity

P.A5.03.11
PD1 ligand regulation during viral infection: Primus inter pares
M. Raffety, J. Hofmann, G. Schönnich, Institut für Virologie, Berlin, Germany.
Enhancement of the immune response to tumours and infections by blocking inhibitory co-stimulation has become an established success. In particular blocking of the CD28 family member PD1 on T cells and its ligands, PD-L1 and PD-L2, has proven therapeutically effective. The regulation of these ligands, however, has not yet been fully elucidated although cytokine and interferon expression is known to be induced by PD1 to PD-L2 in response to stimuli we exposed primary cells (fibroblasts, endothelial cells, dendritic cells and PBMC) to different interferons and PAMPs as well as to active viral infections. Cells were analysed by flow cytometry and functional assays. Comparison between Type I, II and III interferons showed a surprising divergence between otherwise similar cytokines, ranging from no induction (III) to strong (II). Similarly, some viral PAMPs showed strong induction whereas others such as Rigi-I appeared to be ineffective. Viral infection in general rapidly induced PD1 expression. This might argue for a role in the innate immune response to viral infection in addition to downregulation of the adaptive immune response as has been previously demonstrated. In agreement with this was the association of bystander activation with PD1 expression in response to primary infection of PBMC. We propose that the PD1 system has a role to play in innate as well as adaptive immunity.

P.A5.03.12
Alum induces rapid NADPH-oxidase independent NET release in human neutrophils
M. Reithofer1, D. Polak1, C. Zitzmiller1, G. Greiner1, B. Bohle2, B. Jahn-Schmidt2
1 Institute for Pathophysiology and Allergy Research, MCCP PhD Programme, Vienna, Austria, 2 Institute for Pathophysiology and Allergy Research, Vienna, Austria, 3 Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria, Vienna, Austria.
Alum is the most widely used adjuvant, though the mechanism behind its adjuvancy is not totally solved. In mice, host-derived DNA has been reported to be involved in the adjuvant effect of alum. Neutrophils are the first cells at the site of injection in response to strong or particulate stimuli neutrophils have the ability to simultaneously release extracellular DNA and granular material, so-called neutrophil extracellular traps (NETs) which are able to trap and kill microbes. Here we investigated alum-induced NET formation in human neutrophils and its underlying pathway. Neutrophils were stimulated with alum or PMA and ionomycin as positive controls. Strong NET formation was induced by all stimuli as visualized by fluorescent microscopy showing co-localization of extracellular DNA and different granular proteins. In addition, alum-induced neutrophil elastase activity was found in supernatants. Inhibition of downstream signalling molecules by using a plate-reader assay to quantify released DNA were performed, to reveal the pathway underlying NET formation. Ionomycin and alum-induced mitochondrial reactive oxygen species (mROS), whereas PMA triggered cytotoxic NADPH oxidase-dependent ROS. Alum induced rapid DNA release similar to ionomycin and dependent on phagocytosis, extracellular calcium and NFκB signalling. Furthermore, a significant dependence on necroptosis signalling similar to crystal-induced NET release was found. During the process of NET formation, increased glycolysis, as well as mitochondrial respiration was observed. Together, alum potently induces a rapid mROS dependent NET-release in human neutrophils in vitro, utilizing energy from glycolysis and mitochondrial respiration. These NETs may represent danger-associated molecular patterns involved in the initial immune response to alum-activated vaccines.

P.A5.03.13
Distinct roles for Btk in the formation of the B cell immune synapse
S. Roman-Garcia1, S. R. Gardeta2, S. V. Merino-Cortes2, M. J. de Brujin1, R. W. Hendriks2, Y. R. Carrasco1
1 CSIC, Madrid, Spain, 2 Erasmus University Medical Center, Rotterdam, Netherlands.
Bruton's tyrosine kinase (Btk) has a key role in the signaling pathways of receptors essential for the B lymphocyte response. Given its implication in B cell-related immunodeficiencies, leukemias/lymphomas and autoimmuneities, Btk is studied intensely and is a target for therapy. Here we report distinct roles for Btk in antigen-triggered immune synapse (IS) formation of mouse primary B cells. Btk recruitment to the plasma membrane regulates the B cell ability to trigger IS formation as well as its appropriate molecular assembly, Btk shuffling/scaffold activities seem more relevant than the kinase function on that. Btk-kinase activity controls antigen accumulation at the IS through the PLCγ2/Ca²⁺/CaM axis. Impaired Btk membrane recruitment or kinase function likewise alters antigen-triggered microtubule-organizing center (MTOC) polarization to the IS, B cell activation and proliferation. We also show that, for B cell function, IS architecture is as important as the quantity of antigen that accumulates at the synapse.

P.A5.03.14
IL-10 competence and production in murine B cells: find the differences!
S. Tomori1, F. Miao2, J. Dong1, H. Chang1, M. Colombo1, E. Dalla3, A. Radbruch4, C. E. Puccillo5
1 University of Udine, Udine, Italy, 2 German Rheumatism Research Center (DRFZ), Berlin, Germany, 3 Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy.
Under physiological conditions the immune system is maintained under homeostasis thanks to the balance between the regulatory and the effector compartment. Regulatory cells can be detected among several immune populations: B cells with regulatory functions have been described, but still no transcription factor to identify them has been discovered. Our working hypothesis is that we can distinguish between IL-10-competent B cells and IL-10-producing B cells, where the first class is ready for IL-10 production immediately after stimulation, while the others are instructed by the surrounding environment. After 48 hours of stimulation IL-10 production can be induced by infective stimuli, such as LPS or CpG, but not by immune-mediated like through CD40. Very interestingly, if cells that are pre-stimulated through CD40 receive a second stimulus they start transcribing IL-10: these cells are competent for IL-10 production. Of note, the same concept can be applied in the ex vivo situation. Indeed, among total murine splenic B cells stimulated for 5 hours with LPS, PMA and ionomycin, only the 2-3% is able to immediately produce IL-10 and these are genuine IL-10 competent B cells. We set up a method to isolate them at very high purity taking advantage of an IL-10 secretion assay combined with FACS-sorting. On these two populations we performed several analysis, trying to understand which are the mechanisms at the basis of IL-10 production. We firstly analysed the timing of IL-10 production and then compared the transcriptomic signature of IL-10-competent and non-competent B cells with the public database ImmGen.

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POSTER PRESENTATIONS

P.A5.03.15

Activation of the cGAS-STING pathway by chitosan requires the engagement of Dectin-1

J. L. Turley,
Trinity College Dublin, Dublin 2, Ireland.

The cationic polycationic chitosan is an effective adjuvant that induces humoral immunity and Th1 cell responses following vaccination by intramuscular or mucosal routes, supporting its application as an alternative for alum for vaccines that promote cell-mediated immunity.

We previously reported that chitosan promotes dendritic cell (DC) maturation by inducing type I interferons and enhances antigen-specific Th1 responses in a type I IFN receptor-dependent manner. Here we show this response is also dependent on cGAS/STING sensing, mitochondrial disruption, subsequent mtDNA release and subsequent STING pathway activation. We hypothesize that chitosan binds to the STING-1 receptor leading to the activation of phospholipase Cγ2 (PLCγ2) and the flux of calcium from the endoplasmic reticulum (ER). A localised actin polymerisation event then brings the ER and mitochondria into close proximity, allowing a disproportionate amount of calcium to flow into the mitochondria. High calcium levels in the mitochondria result in increased ROS production and mitochondrial depolarisation, likely contributing to the subsequent release of mtDNA. These data indicate that the immunomodulatory properties of chitosan result from this calcium-driven ROS production. This work provides evidence for the first time of a link between CTL receptors and intracellular nucleic acid receptors leading to dendritic cell maturation and enhanced cellular immunity.

P.A5.03.16

Selective recruitment of CD8+ T cells against a novel 12-mer A*68:01-restricted influenza peptide reflects the importance of HLA and TCR profiles

E. B. Clements1, E. C. van de Sandt1, E. Grant1, S. Grant1, J. Rossjohn1, W. Chen1, K. Kedzierska1, 2
University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia, 2Sanquin Blood Supply Foundation, Amsterdam, Netherlands, 3Monash University, Melbourne, Australia, 4La Trobe University, Melbourne, Australia.

Influenza A viruses are responsible for seasonal epidemics and sporadic pandemics that result in significant health, social and economic costs worldwide. In contrast to strain-specific antibodies, immunoproteins provided by CD8+ T cells is long-lasting and cross-strain specific, making it an attractive target for novel universal one-shot vaccine strategies.

We recently identified a novel 12-mer peptide from influenza A virus NP protein (NP145) restricted by HLA-A*68:01. To determine the potential for NP145-specific CTL to contribute to anti-influenza immunity, we dissected the characteristics of this response in A*68:01+ individuals (0-25% allele frequency, depending on ethnicity). We observed NP145-specific responses in ~50% of individuals, ranging from immunodominant to subdominant in magnitude. Remarkably, we did not observe this to result to the same extent of the peptides that were not encoded in the sequence.

Crystal structure analysis of the A*68:01-NP145 complex showed that the central region of the NP145 peptide is highly flexible and may present a difficult target for TCR recognition, especially as A*68:01 does not ligate CD8 for enhancement of TCR-pMHC binding. Single-cell multiplex RT-PCR analysis of TCRβ heterodimers signatures characterized by long CDR3α and -β loops. Our data highlight the role of individual HLA profiles and intrinsic CD8+ T cell quality in determining recruitment of effective epitope-specific responses during infection.

P.A5.03.17

Bystander T-cells support clonal T-cell activation by controlling the release of dendritic cell-derived immune-stimulatory vesicles.

M. Lindenbergh1, D. Koerhuis1, T. Drieskens1, R. Wubbaits1, W. Steurop1, M. Boes2
1Utrecht University, Utrecht, Netherlands, 2UMC-Utrecht, Utrecht, Netherlands.

Extracellular vesicles (EVs) that are released by immune cells are studied intensively for their functions in immune regulation and are scrutinised for their potential in human immunotherapy, for example against cancer. In our search for signals that stimulate the release of functional EVs by dendritic cells (DC) we co-cultured human monocyte-derived DC (moDC) with fixed autologous T-cells. LPS-activated moDC changed their morphology characteristics in response to activated bystander T-cells, while non-activated bystander T-cells had no effect. Exposure of moDC to activated bystander T-cells stimulated the release of moDC-derived EV-associated proteins, including CD9, CD63, CD81, HLA class I, and ICAM-1, although these effects were highly variable between donors, and significant increases could be established only for CD63 and ICAM-1. The release of small RNA profiles was strongly increased upon interaction with activated bystander T-cells, specifically miR155a, known as a central modulator of T-cell responses, was highly increased in EVs released by moDC. Functionally, we observed that EVs from moDC licenced by activated bystander T-cells displayed an enhanced capacity for antigen-specific T-cell activation. Taken together, these results suggest that non-cognate interactions between DC and bystander T-cells can modulate the activity of antigen-specific T-cell responses via EVs.

P.A5.03.18

Leukocyte iRhom2 regulates basal cardiac function

P. A5.03.19

HSPCs prevent chronic stress-induced immune suppression

D. Yin1, H. Zhang1, Y. Z. Caudle2, A. Qin1
1ETSU College of Medicine, Johnson City, United States, 2Xiangya School of Pharmaceutical Sciences, Central South University, Changsha, China.

Introduction. Chronic stress has been demonstrated to play a significant role in the development and progression of cardiovascular diseases (CVDs). Chronic stress can modulate the immune system, leading to the dysregulation of immune responses that contribute to the development of CVDs.

Methods. We used a chronic restraint stress model, where male C57BL/6 mice were subjected to a 5-week period of restraint stress. Baseline and stress-induced changes in body weight, food intake, and heart rate were monitored. Serum levels of cytokines, including IL-1β, IL-6, TNF-α, and IFN-γ, were measured to assess the immune response to chronic stress. The expression of the genes involved in the stress response, such as the stress-inducible protein 1 (Sip1), was also measured.

Results. We found that chronic restraint stress led to a significant increase in the expression of Sip1 and the stress-induced cytokines in the heart tissue. The expression of genes involved in the immune response, such as the immunosuppressive cytokine IL-10, was also increased in the heart tissue, indicating a reduced immune response.

Conclusions. Our results suggest that chronic restraint stress leads to a significant increase in the expression of stress-induced cytokines and genes involved in the immune response. These changes may contribute to the development of CVDs.

P.A5.03.20

Dysfunctional proteolysis of CD74 by SPP2 alpha promotes response towards CD74 that is specific for ankylosing spondylitis and rheumatoid arthritis

T. van Kempen1, E. Leijten1, M. Lindenbergh1, M. Olde Nordkamp1, C. E. van de Sandt1, J. Sanchez, A. Gutierrez Del Arroyo, G. Ackland, S. Maleux, K. Traulfsen, D. Koerhuis2
1UMC-Utrecht, Utrecht, Netherlands, 2Utrecht University, Utrecht, Netherlands, 3Cantonal Hospital St. Gallen, St. Gallen, Switzerland, 4Medical University Hannover, Germany, 5Queen Mary University London, London, United Kingdom.

Ankylosing spondylitis (AS) is a chronic inflammatory disease that affects the axial skeleton, particularly the spine and sacroiliac joints. The disease is characterized by chronic inflammation of the joints, leading to the formation of bone and in rare cases, spinal fusion. The disease is associated with a genetic predisposition, with the human leukocyte antigen (HLA) B27 allele being the most common risk factor. However, the pathogenesis of AS is not fully understood, and the mechanisms underlying the disease remain elusive.

In this study, we investigated the role of the major histocompatibility complex (MHC) class II molecule CD74 in the pathogenesis of AS. CD74 is a transmembrane protein that is expressed on the surface of cells and is involved in the presentation of peptides to T cells. The proteolysis of CD74 is a critical step in the T-cell response, and alterations in this process can lead to the development of autoimmune diseases.

We found that the proteolysis of CD74 is dysregulated in AS patients, with an increased expression of the protease SPP2 alpha (SPP2α). This protease is known to target CD74 and promote its proteolytic cleavage. In AS patients, the activation of SPP2α is increased, leading to the dysregulation of the CD74/cytokine network, which is crucial for the pathogenesis of AS.

Our findings suggest that the dysregulation of the CD74/protease network is a key mechanism in the development of AS. Targeting this pathway could represent a novel therapeutic strategy for the treatment of AS. Further studies are needed to confirm these findings and to explore the potential of SPP2α inhibitors as a therapeutic intervention for AS.

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P.A.S.04.01

Transcriptional regulation of the IL-2 gene through Tip60 acetyltransferase binding in the ARRE-2 enhancer element
I. Aggeletopoulou, I. Panagoulia, F. Karagiannis, P. Davoulou, T. Georgakopouloas, A. Mouzaki
Laboratory of Immunohematology, Division of Hematology, Department of Internal Medicine, Faculty of Medicine, University of Patras, Patras, Greece.

Introduction: Acetyltransferase Tip60 regulates gene transcription by interacting with promoter binding factors. Our previous publication has shown that IL-2 expression is blocked by a deletion of the ARRE-2 (IκBα motif), that binds to the ARRE-2 element of the IL-2 promoter without physical interaction with NFAT-2, which binds to the same element promoting IL-2 activation. In this work, we studied the role of Tip60 on the IL-2 regulation through its possible interactions with Ets-2 and NFAT-2. Methods: Tip60, Ets-2 and NFAT-2 interactions were investigated by co-immunoprecipitation experiments in Jurkat cells in the absence (CM) or presence of the mitogens phorbol myristate acetate and ionomycin (P/Ι). Co-localization of Tip60, Ets-2 and NFAT-2 in Jurkat cells (P/Ι) was investigated by co-immunofluorescence. ChiP analysis was performed to determine Tip60 and Ets-2 binding to the IL-2 promoter. Results: Tip60 overexpression and silencing resulted in the activation and suppression of the IL-2 gene, respectively. Tip60 and Ets-2 interaction and co-localization was observed in CM and P/Ι conditions whereas Tip60 and NFAT-2 interaction was observed in P/Ι conditions only. In unstimulated cells, both Tip60 and Ets-2 bound to the ARRE-2 region. In contrast, P/Ι stimulation resulted in the departure of Tip60 and Ets-2 from ARRE-2 and the binding of Tip60 to the core promoter.Conclusion: Tip60 interacts with Ets-2 in both CM and P/Ι and with NFAT-2 in P/Ι conditions. We suggest that Tip60 contributes to the IL-2 transcriptional activation by dissociating Ets-2 from its binding site and permitting NFAT-2 binding to the ARRE-2 element.

P.A.S.04.02

Aurora A function in CD8 killing activity
A. Alcaraz-Serna, E. Bustos-Morant, N. Blas-Rus, S. Iborra, F. Sanchez-Madrid
1Servicio de Immunologia, Hospital Universitario La Princesa, Universidad Autonoma de Madrid, Instituto Investigacion Sanitaria La Princesa (IIS-IP), Madrid, Spain, 2Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.

Aurora A has been studied in cell cycle progression and tumor generation. Recent work has revealed an unexpected function of Aurora A during inflammation and graft versus host disease development. However, the role of Aurora A in CD8+ T cell effector function and its cytotoxic T lymphocytes-mediated antiviral response has not been explored. To study the role of Aurora A in the CD8 lymphocyte cytotoxic function regulation, an in vitro study has been performed by flow cytometry analysis of CD107 surface expression and cytotoxic assays. Additionally, an in vivo analysis was conducted by infecting mice adoptively transferred with OT-I CD8 T cells and challenged with a Vaccinia-OVA infection in the presence of a specific drug inhibitor of Aurora A. Aurora A inhibition leads to an impairment either on the peptide-specific cytotoxicity and on the degranulation ability of CD8+ T cells. Finally, in an in vivo model of Vaccinia infection we have proved that Aurora A is necessary for the MHC-I restricted CD8+ T cells-mediated antiviral response. We can then conclude that Aurora A activity is a key factor for the cytotoxic T lymphocytes effector function and also for its action against a viral infectious threat.

P.A.S.04.03

The role of DNA damage response in modulating functional plasticity of macrophages
A. Bansal, B. Schumacher
CECAD Research Center, University of Cologne, Cologne, Germany.

Introduction - The key innate immune players like neutrophils and macrophages generates potent genotoxic species (ROS and NOS) during infection and tissue injury, suggesting interplay and overlap between DNA damage response (DDR) and innate immune response. However, molecular mechanisms linking DDR with innate immune response are still poorly understood. Therefore, we addressed the following questions: 1) Does DDR prime the macrophages, thus predefining immune response to forthcoming stimuli? 2) What are the key molecular players and signaling pathways underlying the interplay of DDR and innate immune response? Materials and Methods - Macrophages were primed with low dose of UV light, which causes moderate and uniform DNA damage. Henceforth, UV primed macrophages are referred as “MUV”. To define MUV activation and polarization, immune phenotyping, seahorse metabolic energetic analysis and in vitro functional assays (endotaxin tolerance assay, phagocytosis and gap closure assay) were performed. The phagophagocytosis reactions was carried out to identify key molecular players linking DNA damage and immune response of MUV cells. Results - The MUV cells express upregulated actin remodeling genes and have alerted metabolic energetics. Our, in vitro functional assays demonstrate that MUV cells are endotaxin tolerant, have increased phagocytosis capacity and secrete cytokines which increases endothelial cell migration. The phagophagocytosis data analysis further suggests, the possible role of DDR-induced histone modification and reprogramming in functional gain of MUV macrophages. Conclusions Collectively, our findings show that DNA damage response activation is sufficient to prime the macrophage and modulate its immune function.

P.A.S.04.04

Recurrent aphthous stomatitis and role of metals in the etiology
1Biomedical Research Institute of Medical Research, Charles University in Prague, Czech Republic, 2Institute of Medical Research, Academy of Sciences of the Czech Republic, Prague, Czech Republic, 3Institute of Medical Research, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Recurrent aphthous stomatitis (RAS) is the most common disease of oral mucosa, affecting 20-25 % of population worldwide. Nevertheless its etiopathology remains unexplained. This condition is characterized by multiple recurrent ulcers with circumscribed margins and yellow floors. RAS is considered to be a multifactorial disorder, the immune and genetic predisposition are the leading hypothesis. In our study the role of metals in RAS pathogenesis was evaluated. In total, 54 patients with recurrent aphthous stomatitis and 54 age-matched control volunteers were enrolled. Metal levels were measured in blood plasma and clinical scores were evaluated with modified RAS activity index. A significant difference was found between RAS patients and controls in elements such as copper, zinc, cadmium, lead and chromium. Our findings, suggest that the role of metals is an important factor in the development of RAS.

P.A.S.04.05

A mobile eukaryotic network connects clathrin-independent receptor endocytosis to recycling and promotes T cell activation
E. B. Compeel, K. Kraus, M. Ecker, N. Rother, R. Nicovich, J. Lou, H. Vartoukian, J. Gaus, J. Rossy
1University of Oxford, Oxford, United Kingdom, 2University of New South Wales, Sydney, Australia, 3Monash Biomedicine Discovery Institute, Melbourne, Australia, 4Garvan Institute of Medical Research, Sydney, Australia, 5University of New South Wales, Sydney, Australia, 6Commonwealth Scientific and Industrial Research Organisation, Geelong, Australia, 7Biotechline Institute Thurgau, Thurgau, Switzerland, 8Biotechnology Institute Thurgau, Thurgau, Switzerland.

Endocytosis of surface receptors and their polarized recycling back to the plasma membrane are central to many cellular processes, such as cell migration, cytokinesis, basolateral polarity of epithelial cells and T cell activation. Little is known about the mechanisms that control the organization of recycling endosomes and how they connect to receptor endocytosis. Here we followed the endocytosis of the T cell receptor (TCR), from internalization at the plasma membrane to recycling back to the immunological synapse. We showed that TCR triggering leads to its rapid uptake through a clathrin-independent pathway. Immediately after internalisation, TCR is incorporated into a mobile and long-lived endosome that we marked by the membrane-organising proteins flotillins. Although flotillins are not required for TCR internalization, they are necessary for TCR recycling to the immunological synapse, TCR its nanoscaled spatial surface distribution, TCR signalling, and efficient primary T cell activation. Collectively, our data supports a model in which a novel endocytic sorting machinery underpinned by flotillins promotes the recycling of internalized TCR complexes to the immunological synapse to coordinate TCR nanoscaled organization that supports efficient T cell activation.

P.A.S.04.06

High arginase-1 levels in neonatal monocytes interfere with bactericidal functions and production of cytokines
S. Dreschers, K. Ohl, C. Pfenner, K. Tenbrock, T. Orlikowsky
Children’s University Hospital Aachen, Aachen, Germany.

Introduction: Bacterial infections enhance serum levels of arginine due to protein catabolism. Monocytes and monocytes derived macrophages (MDM) express the enzyme arginase-1 and INOS which metabolize arginine. Activity of iNOS and arginase-1 results in opposing effects: either the pro-inflammatory response (NO and ROS-production) and enhanced bacterial inactivation or an anti-inflammatory response via production of IL-10 and expansion of Th2-like T-cell subsets. Controlling the arginine metabolism can be predetermining the course of a bacterial infection. Hypothesis: High arginase-expression in MDM of newborn macrophages (I2BM) strengthens anti-inflammatory responses compared to FRBM.

P.A.S.04.07

Endocytosis of surface receptors and their polarized recycling back to the plasma membrane are central to many cellular processes, such as cell migration, cytokinesis, basolateral polarity of epithelial cells and T cell activation. Little is known about the mechanisms that control the organization of recycling endosomes and how they connect to receptor endocytosis. Here we followed the endocytosis of the T cell receptor (TCR), from internalization at the plasma membrane to recycling back to the immunological synapse. We showed that TCR triggering leads to its rapid uptake through a clathrin-independent pathway. Immediately after internalisation, TCR is incorporated into a mobile and long-lived endosome that we marked by the membrane-organising proteins flotillins. Although flotillins are not required for TCR internalization, they are necessary for TCR its recycling to the immunological synapse, TCR its nanoscaled spatial surface distribution, TCR signalling, and efficient primary T cell activation. Collectively, our data supports a model in which a novel endocytic sorting machinery underpinned by flotillins promotes the recycling of internalized TCR complexes to the immunological synapse to coordinate TCR nanoscaled organization that supports efficient T cell activation.
Material and methods: Polarization of monocyte-derived MΦ from cord blood and from adult peripheral blood (PBMD) according to published protocols. FACs-based imaging revealed that receptors and mediators responsive to IL-36α and/or IL-36γ (both IL-36α and IL-36γ are expressed as inactive precursors that must undergo proteolytic truncation to become biologically active. Initial experiments incubating IL-36γ in pathogenic bacterial and fungal conditioned media illustrated IL-36γ is susceptible to cleavage by pathogen-derived proteases. Importantly, analysis by mass spectrometry showed Aspergillus fumigatus (Af), Trichophyton an inactive precursor that must undergo proteolytic truncation to become biologically active. Most testing of IL-36γ was conducted with Af. M1-CBMΦ produced less NO than M1-PBMΦ. All MΦ subsets featured equal phagocytosis-capacity. Intracellular killing of Candida was unaltered. M2-CBMΦ ROS production was lowered compared to M2-PBMΦ. Conclusion: Overexpression of Arginase-1 in CBMΦ, is attributed to stronger pro-inflammatory responses. Bacterial functions were unaffected.

P.A5.04.07 A key role for microRNAs in regulating IL-17 versus IFN-γ production by yG T cells T. Amado, N. Schmoll, D. Sabaté, F. Enguita, D. Inácio, B. Silva-Santos, A. Gomes
1Instituto de Medicina Molecular, Lisbon, Portugal, 2Instituto Gulbenkian de Ciência, Deiras, Portugal, 3Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal.

γδ T cells are an important source of the pro-inflammatory cytokines IFN-γ and IL-17 in (patho)physiologic conditions. In the mouse, CD27+ γδ T cells are committed to IFN-γ expression, whereas their CD27- counterparts may IL-17 but are capable of co-expressing both cytokines. γδ T cells under inflammatory conditions. We aim to characterize a novel layer of microRNA-mediated regulation of effector γδ T cell differentiation. First, by comparing the microRNA pools of the two CD27+ based γδ T cell subsets, we found that the expression patterns in γδ T cells and their restricted IFN-γ production by targeting Nod1 mRNA. Next, to overcome the caveat of using surface markers, which do not allow isolation of pure populations of IL-17 or IFN-γ-producing γδ T cells, we used a double reporter IL-17-GFP; IFN-γ-YFP mouse strain.

Pure IL-17+ or IFN-γ+ γδ T cell populations were isolated from peripheral lymphoid organs and subjected to next generation sequencing analysis of both microRNA and mRNA repertoires. This allowed us to identify, for the first time, microRNA and mRNA signatures directly associated with cytokine expression, rather than TCR Vγ usage or maturation markers. Furthermore, differentially expressed microRNAs and mRNAs were bioinformatically integrated into networks that allowed the identification of microRNAs predicted to target key determinants of the IL-17 program and mRNA candidates for the IFN-γ program of γδ T cells. Ongoing molecular assays provide an unprecedented functional characterization of the impact of microRNAs on the identity and differentiation of effector γδ T cell subsets.

P.A5.04.08 Expression of endogenous interleukin-36 activity A. Jasfar, M. Nicklin
University of Sheffield, Sheffield, United Kingdom.

INTRODUCTION: IL-36 cytokines comprise three agonists, IL-36α, IL-36β, IL-36γ, and antagonist IL-36Ra. IL-36 appears to be an important mediator for inflammation and immunity, particularly in the skin and other epithelia.

Material and methods: We used rhIL-1α, rhIL-36α and rhTNF at close to saturating concentrations to activate the IL-36 genes. IL-36 mRNAs were detected in both HaCaT (an untransformed human keratinocyte line) and HT-29 (a human colorectal carcinoma/epithelial cell line).

RESULTS: RT-PCR and RT-qPCR showed that IL-36α, IL-36β, IL-36γ, and IL-36Ra mRNAs were expressed in HaCaT in monolayer by Ca2+ modulation had little effect on the inducibility of IL-36β or IL-36γ. IL-36α, IL-36γ and IL-36Ra mRNAs were also expressed by HT-29 but the standardised levels of IL-36β and IL-36γ were an order of magnitude lower compared with HaCaT. In HaCaT, the inducer cytokines were effective in the order TNF-α>IL-1β>IL-6. In HT29 IL-36 was more effective than IL-17. In HaCaT also responded to inducers of IL-8/CXCL8 secretion in the order TNF-α>IL-1>IL-6. In HT-29, IL-8 secretion was very effectively induced by rhIL-36. CONCLUSION: Expression levels of IL-36β and IL-36γ seem to be intrinsically cell line dependent. Maximum expression in HaCaT was an order of magnitude stronger than in HT-29. IL-36 expression was equal across all cell lines.

P.A5.04.09 Investigating the post-transcriptional cooperation of Roquin and Regnase-1 in T cell differentiation N. Kronbeck, G. Cabaò, R. Zimmer, V. Heissmeyer
1Biomedical Center Munich, Ludwig-Maximilians-Universität, München, Germany, 2Department of Informatics, Ludwig-Maximilians-Universität, München, Germany.

Gene regulation on the post-transcriptional level exerts essential control over immune control responses and is needed to prevent autoimmune diseases. The RNA-binding proteins Roquin-1 and its paralog Roquin-2 were found to be essential for the prevention of autoimmunity and autoinflammation by controlling T cell activation and differentiation. Roquin protein-mediated NO production was comparable with the cytoplasmic domain of a high affinity/avidity and induce mRNA decay post-transcriptionally. In T cells, the expression of Roquin proteins themselves is regulated by proteolytic MALT1 cleavage downstream of the T cell receptor in a signal strength-dependent manner. The endonuclease Regnase-1 is regulated in the very same way by MALT1 cleavage. Moreover Roquin proteins and Regnase-1 share a common set of mRNA targets and can cooperate in the regulation of mRNAs.

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Furthermore, differentially expressed microRNAs and mRNAs were bioinformatically integrated into networks that allowed the identification of microRNAs predicted to target key determinants of the IL-17 program and mRNA candidates for the IFN-γ program of γδ T cells. Ongoing molecular assays provide an unprecedented functional characterization of the impact of microRNAs on the identity and differentiation of effector γδ T cell subsets.

P.A5.04.10 The role of dendritic cells in lactobacilli-mediated damping of pro-inflammatory immune responses G. Lasavvicate, J. Quin, A. Östlund Farrants, E. Sverremark-Ekström
Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden.

During the last year, research has focused on the interplay between the microbiota and the immune system. Lactobacilli, which are frequently found in the human gut, are of particular interest due to their beneficial probiotic properties. Recent results from our group and others suggest that lactobacilli modulate T-cell responses in vitro, however, the mechanisms involved are not yet fully understood. Since antigen presenting cells (APCs) like dendritic cells (DCs) are responsible for immune activation, we are interested in whether lactobacilli induce epigenetic changes in DCs and influence their functional phenotype. Our preliminary results show that stimulation of monocyte-derived DCs with Lactobacillus (L.) reuteri cell free supernatant (CFS) induce maturation of DCs in terms of increased cell surface and gene expression of CD83. We also show that the expression of pro-inflammatory cytokines IL-6 and IL-23 is upregulated in L. reuteri-CFS stimulated cells. Further, the results from chromatin immunoprecipitation (ChIP) experiments show that chromatin accessibility at the promoter region of genes encoding markers important for DC maturation and function clearly differs between L. reuteri-CFS and Staphylococcus aureus-CFS stimulation. To date, we hypothesize that changes in histone modifications, which can be identified by “activating and silencing marks”, and recruitment of different transcription factors at the promoter region of DCs genes might contribute to lactobacilli-mediated immune regulation.

P.A5.04.11 IL-36 is activated by several pathogen-derived proteases and thus functions as a global alarm of epithelial infection J. Alsinaough, T. Macleod, M. Stacey, M. Wittmann
1University of Leeds, Leeds, United Kingdom, 2Institute of Rheumatic and Musculoskeletal Medicine, Leeds, United Kingdom.

The interleukin (IL)-1 family of cytokines are fundamental regulators of the innate immune system, pivotal in initiating and orchestrating inflammation. IL-36γ is a recently described member illustrated IL-36γ is susceptible to cleavage by pathogen-derived proteases. Importantly, analysis by mass spectrometry showed Aspergillus fumigatus (Af), Trichophyton rubrum (Tr) and Streplococcus pyogenes (Sp) cleave IL-36γ to its potent pro-inflammatory truncation (IL-36γ S18). Subsequent experiments utilising protease-knockout strains and recombinant proteases demonstrated IL-36γ activation is mediated by virulence factors secreted by Af and Sp. Challenging oral epithelial cells with heat fixed Af and Sp increased expression of IL-36γ, whilst addition of five pathogens caused both IL-36γ release and activation. Furthermore, IL-36γ activation was inhibited when experiments were repeated with protease-knockout strains. In summary, these investigations show invasive bacterial and fungal pathogens in vitro, overexpress IL-36γ activity, which activate the cytokine once it has been released. Given that IL-36γ is activated by such a broad range of pathogen-derived proteases, it is postulated this cytokine functions as a sensor of exogenous protease activity, thus being a broad alarm of infection. Ultimately, this mechanism may represent an essential mediator of host defence against a variety of important human pathogens.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands.
Chemokines and their receptors are key molecules that coordinate trafficking of lymphocyte subtypes. CCR5, the chemokine receptor for the ligands CCL3, CCL4 and CCL5, is also a coreceptor that is necessary for maximal stimulation of the T cell receptor (TCR) and CCR5 ligand secretion by both the antigen-presenting cell and the T-cell. Although the role of CCR5 in naive CD4+ T cell activation is well-established, whether it has additional functions in antigen-experienced T cells remains unknown. The TCR is organised in nanoclusters, which are larger in activated and memory than in naive counterparts. This reorganisation to larger TCR oligomers explains the increased antigen sensitivity of preactivated T-cells, probably because the nanoclusters can be stimulated at lower antigen concentrations than monomeric TCRs. We report that CCR5 activity determines TCR nanocluster size and valency in antigen-experienced T cells. This activity is CCR5+ lymphocyte-specific and independent of CCR5-induced costimulatory signals. CCR5-induced TCR nanoclustering was associated to changes in cell lipid composition. Activated CCR5+ CD4+ T cells had higher ceramide levels than CCR5- counterparts, coinciding with increased expression of several ceramide synthase isoforms in the CCR5+ cells. In splenomegaly-induced treated live cells and in artificial liposomes, ceramide levels critically determined TCR nanocluster size. Finally, CCR5 deficiency impaired antigen sensitivity of antigen-experienced CD4+ T cells in vitro, and reduced T-cell help for immunoglobulin class switching in vivo. Our results identify a CCR5 role in TCR nanocluster formation and CCR5 participation in memory T-cell function.

The aim of the study was to examine the expression level of type 1 and 2 receptors to TNF-alpha (TNFR1/TNFR2) on main immune subsets of peripheral blood cells. Methods: The study was conducted on 46 healthy donors (18-77 years). Co-expression and number of TNFR1/2 were calculated for monocytes, B-cells, T-cells, as well as among: T-helper cells, T-helpers, activated CD8+ and CD4+ cells, memory T-cells and naive T-cells, and regulatory cells by flow cytometry analysis (BD FacsVerse, USA). Results: The highest percentage of double positive cells was in activated cytotoxic T-cells subset (25.8%), slightly lesser - in monocytes (15.2%), cytotoxic T-memory cells (14.3%) and CD5+ B-cells (13.9%), total B-cell pool and activated T-helper (11.5%); for the remaining subpopulations, proportion of double-positive cells did not exceed 10%. For all populations studied, small fraction of cells expressed only type 1 receptors (less than 0.5%) was identified for T-regulatory cells, cytotoxic and T-regulatory memory cells and CD5+ B-cells. Conversely, the proportion of cells expressing only type 2 receptors to all studied populations was more than 25% and at least twice the proportion of double-positive cells. Conclusion: The distribution of TNFR1/2 differed significantly among the main immune subsets, which can lead to different levels and types of cell response to cytokine. Simultaneous presence of both types of receptors on the cell surface is associated with increase of their expression density.
Transcription factor Ets-2 is involved in diverse biological functions and transcriptional regulation. Recent work in our laboratory has shown that in naive T helper cells, Ets-2 mRNA overexpression is confirmed...
Results: Overexpression of Ets-2 in Jurkat and H938 cells induced NFATc2, NF-kB p65 and c-jun protein levels under both CM and P/I conditions. In unstimulated H938 cells, overexpression led to a reduction in CD10 levels, whereas in stimulated cells Ets-2 overexpression led to a redshift. In unstimulated HEK cells, increasing amounts of pCDNA-ets2 led to an increase in c-jun and CDKX10 levels; in contrast, in stimulated HEK cells resulted in a reduction in CDKX10 and c-jun levels. Conclusion: Ets-2 is involved in the regulation of expression and synthesis of key lymphotropic factors. Our results set the stage for further studies to elucidate the roles of Ets-2 in the regulation of signaling pathways involved in the activation and differentiation of B and T lymphocytes.

P.AS.05.04 Antigen dependent TCR repertoire relation of human cTRIF- and non-cTRIF- CD4 T-cells

M. Hu1, A. Cassattta1, A. Lanzaaveti1, S. Salustro1;

1-Institute of Immunology, ETH Zurich, Zurich, Switzerland, 2-Institute for Research in Biomedicine, Bellinzona, Switzerland.

Abstract: The work also provides new insights into the role of IL-33/ST2 pathway in iron homeostasis. We characterized two microenvironmental cues for the differentiation of a tissue-resident macrophage subset, and it is example of a local cytokine-dependent functional specialisation of a tissue-resident RPM. Thus, reconstitution of RPM-deficient mice with monocyte-derived RPM, with a profound phenotypic alteration of the remaining RPM. Consequently, aging ST2−/− mice displayed high levels of SpiC in monocyte-derived macrophages, and promotes the generation of mature RPM. Mice deficient for the IL-33 receptor ST2 display a cell-autonomous deficit in macrophages, and promotes their differentiation into a precursor pre-RPM phenotype (CD11bhi F4/80−). While SpiC transcription factor is overexpressed in monocyte-derived macrophages, and regulates the expression of the IL-33 receptor ST2. This study will reveal the heterogeneity within cTRIF− and non-cTRIF− cells in terms of antigen specific clonal relations with non-cTRIF−, and its undescribed antigen types dependency.

P.AS.05.05 TCR signaling strength promotes differentiation of human T follicular helper cells

T. Jorritsma1, N. J. Verstegen2,3, P. A. Unger1, B. P. Nicollet1, A. Ten Brinke1, M. van Ham1,2;

1-QuaS Group "Clinical Cell Processing and Purification", Institute for Advanced Study, TUM, Munich, Germany, 2-Department for Infection Research, Technical Universität München, Munich, Germany, 3-German Mouse Clinic, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuhberg, Germany.

Abstract: We previously reported that, in addition to IL-21, human Tfh cells may plastically coexpress the Th1 cytokine IFN-γ and/or the Th2 cytokine IL-4. As IFN-γ directs chemokine expression during B cell differentiation and IL-4 promotes memory B cell formation, it is important to elucidate how cytokine coexpression in Tfh cells is controlled. Here we identified TCR signaling strength as an important regulator of plasticity in the Tfh-like, IL21-producing cell population. IL-21 expression is favored by high TCR signaling in human naive CD4+ T cells. In line with the known preference for Th1 skewing under those conditions, the fraction of IL21-producing cells that coexpress IFN-γ progressively increases with TCR signaling strength. In contrast, coexpression with IL-4 decreases, as also in Tfh cells, IL-4 benefits from low TCR signaling. Similar to the notion that Th1/Th2 polarization is largely mutually exclusive, we demonstrated that the inhibitory effect of hallmark cytokines IFN-γ and IL-4 on Th2 and Th1 differentiation respectively, we demonstrate that IL-4 inhibits generation of Th1-like, IL21-producing cells whereas IL-21 promotes autocrine IL-21 expression, but inhibits expression of the Th2-cytokine IL-4. These data show how the formation of Th1-like, IL21-producing cells modulate cell differentiation by regulating the magnitude of TCR signaling and availability of IL-4 and IL-21, which may be instrumental to improve vaccine effectiveness.

P.AS.05.06 Antigen-dependent cell cycle speed during priming shapes CDB T cell memory

K. Retzschmer1, M. Flossdorf1, M. Plambek1, J. Mir1, A. Taska1, Y. Cho1, I. Treise1, D. H. Busch1,2,3, V. R. Buchholz1,4;

1-Institute for Medical Microbiology, Immunology and Hygiene, Heinrich-Heine Universität Düsseldorf (HHU), Germany, 2-Department for Infection Research, Technical Universität München, Munich, Germany, 3-Department of Medical University of Oxford, Oxford, United Kingdom, 4-Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.

Abstract: Initial antigen encounter triggers T cells to proliferate at speeds close to the physiologic maximum of mammalian T cells. T cell memory, on the other hand, is maintained in absence of antigen by exceedingly rare cell divisions. The transition between these fundamentally different proliferative programs has been difficult to resolve via population-based analyses. Here we combined an approach for mapping the fate of single CDB T cells, developed in our laboratory (I-J, with the timed depletion of peptide-pulsed dendritic cells (DCs) in vivo. Computational modelling and cell cycle analyses showed that long before reaching peak expansion, slower cycling central memory precursors (CMPs) segregated from rapidly dividing effector subsets. Moreover, timed depletion revealed that cycling speed of CMPs was selectively dependent on sustained antigenic stimulation. Accordingly, recall responses were impaired when primary antigen curtailed was curtailed but not when inflammatory stimuli were reduced. By identifying an antigen-dependent hierarchy of cell cycle speeds among emerging CDB T cell subsets, we provide crucial insight into the intrinsic biases important to guide future vaccination strategies and adoptive T cell therapy.

P.AS.05.07 Identification of novel modulators of MR1 trafficking using a gene trap screen in haploid cells

M. Hu1,2,3, M. Flossdorf1, M. Plambek1, J. Mir1, A. Taska1, Y. Cho1, I. Treise1, D. H. Busch1,2,3, V. R. Buchholz1,4;

1-MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom, 2-Ludwig Institute for Cancer Research, Target Discovery Institute, National Institute for Medical Research, University of Oxford, Oxford, United Kingdom, 3-Immunology and Nuclear Organization, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands.

Abstract: MR1 is a non-classical MHC class I molecule expressed on intraepithelial lymphoid T cells and non-cTFH CD4 memory T cells. In line with the known preference for Th1 skewing under those conditions, the fraction of IL21-producing cells that coexpress IFN-γ progressively increases with TCR signaling strength. In contrast, coexpression with IL-4 decreases, as also in Tfh cells, IL-4 benefits from low TCR signaling. Similar to the notion that Th1/Th2 polarization is largely mutually exclusive, we demonstrated that the inhibitory effect of hallmark cytokines IFN-γ and IL-4 on Th2 and Th1 differentiation respectively, we demonstrate that IL-4 inhibits generation of Th1-like, IL21-producing cells whereas IL-21 promotes autocrine IL-21 expression, but inhibits expression of the Th2-cytokine IL-4. These data show how the formation of Th1-like, IL21-producing cells modulate cell differentiation by regulating the magnitude of TCR signaling and availability of IL-4 and IL-21, which may be instrumental to improve vaccine effectiveness.

P.AS.05.08 Identification of novel modulators of MR1 trafficking using a gene trap screen in haploid cells

C. Kulicke1, E. De Zari1, M. Salio1, P. Kerner2,3, S. Nijman1, V. Cerundolo4;

1-MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom, 2-Ludwig Institute for Cancer Research, Target Discovery Institute, National Institute for Medical Research, University of Oxford, Oxford, United Kingdom, 3-Translational Gastroenterology Unit, John Radcliffe Hospital, Headington, Oxford, United Kingdom.

Abstract: The monomorphic MHC-I-related protein MR1 presents bacterial metabolites to mucosal-associated invariant T (MAIT) cells, an innate-like subset of T lymphocytes. The known MAIT-activating MR1 ligands are intermediates of riboflavin synthesis, a pathway specific to certain fungi and bacteria and, thus, intrinsically non-self for humans. Here, we use a functional genetic screening technique based on insertional mutagenesis of the near-haploid human cell line HAP1 to discover novel players in MR1 antigen presentation and trafficking. A HAP1 clone overexpressing MR1 was introduced with a gene trap virus to inactivate genes in an unbiased manner. Subsequently, the mutagenised population was treated with the MR1-stabilising ligand Acetyl-6-Formyltryptophan and stained for MR1 surface expression. The tails of the distribution were FACs sorted to enrich for cells in which positive or negative regulators of MR1 were inactivated. Mapping of the viral insertion sites by Illumina deep sequencing allowed identification of genes statistically overrepresented in either of the two sorted populations which constitute putative modulators of MR1 intracellular trafficking or MR1 stability. The most significant positive regulators of MR1 trafficking demonstrated the enrichment of Candida, Tetanus specific cT1,2 FH cells with GC T1,2 cells. ICOS+ FH1,2 cells displayed antigen dependent pattern. C.Albicans, Tetanus toxoid specific cT1,2 FH cells with ICOS+ FH1,2 cells. ICOS+ FH1,2 cells displayed antigen dependent pattern. C.Albicans, Tetanus toxoid specific cT1,2 FH cells with ICOS+ FH1,2 cells. ICOS+ FH1,2 cells displayed antigen dependent pattern. C.Albicans, Tetanus toxoid specific cT1,2 FH cells with ICOS+ FH1,2 cells. ICOS+ FH1,2 cells displayed antigen dependent pattern. C.Albicans, Tetanus toxoid specific cT1,2 FH cells with ICOS+ FH1,2 cells. ICOS+ FH1,2 cells displayed antigen dependent pattern. C.Albicans, Tetanus toxoid specific cT1,2 FH cells with ICOS+ FH1,2 cells. ICOS+ FH1,2 cells displayed antigen dependent pattern. C.Albicans, Tetanus toxoid specific cT1,2 FH cells with ICOS+ FH1,2 cells. ICOS+ FH1,2 cells displayed antigen dependent pattern. C.Albicans, Tetanus toxoid specific cT1,2 FH cells.
POSTER PRESENTATIONS
P.A5.05.10 The functional role of Delta24PD1-TLR4 pathway in augmenting Vdelta2 T cell stimulation of CD4+ T cell response
Y. Mo1, A. K. Cheung2, Z. Chen2
1AIDS Institute, Research Center for Infection and Immunity, Department of Microbiology, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong SAR, China; 2Department of Biolog, Hong Kong Baptist University, Hong Kong SAR, China.

Immune system plays a crucial role in different diseases directly or indirectly, for instance, acquired immunodeficiency diseases and cancer progression. PD-1 and its ligands, PD-L1 and PD-L2, negatively regulate the immune response via suppressing T cell functions. In contrast, a novel alternatively spliced isoform of human PD-L1, named as Delta24PD1, may substitute for a new immune-stimulator due to its characteristics that it does not bind to either PD-L1 or PD-L2 and that it can bind to TLR4. Our recent study found an increased level of Delta24PD1 protein on Vδ6 subset of Vδ6-T cells in HIV-1 acute patients, which induced intestinal inflammation. To further define the function of Delta24PD1, we show here that activated Vδ6 T cells in vitro elevated Delta24PD1 level while co-upregulated MHC class II expression. Since it was reported that Vδ6 T cells can act as an antigen presenting cell, we hypothesize that Delta24PD1 on Vδ6 T cells could be a role in stimulating T cell responses. By investigating TLR4 level on CD4+ T cells, our data show that the TLR4 could be upregulated upon pan-activation, with particularly significant high expression on the CD45RA+CD45RO+ transitional subset of CD4+ T cells. Furthermore, co-culture experiments of virus-induced Δ24PD1+Vδ6 T cells and autologous effector cells resulted in the CD45R0+CD45RA+ CD4 cells being activated being measured by IFN-γ production, which is impaired when blocking antibody against Delta24PD1 was used. Therefore, our study suggests that the Δ24PD1/TLR4 pathway has a novel niche in stimulating the CD45RA+/CD45RO+ CD4 subset exhibited by Vδ6 T cells.

P.A5.05.11 The environmental exposure is more important than BCG vaccination for the maturation of infant Vγ9Vδ2 T cells
M. Papadopoulou1, T. Dimaras, W. Hanekom2, E. Nemes1, D. Vermijlen1
1Department of Pharmacology and Pharmacotherapeutics, Université Libre de Bruxelles, Brussels, Belgium; 2Institute for Medical Immunology, Université Libre de Bruxelles, Brussels, Belgium; 3South African Tuberculosis Vaccine Initiative, University of Cape Town, Cape Town, South Africa.

γδ T cells are unconventional lymphocytes sharing attributes of both innate and adaptive immunity. Vγ9Vδ2 T cells, which react towards microbe- and host-derived non-peptidic metabolites (phosphoantigens), are the major γδ T cell population in adult human peripheral blood. Some of their main effector functions, such as IFN-γ production, are already programmed before birth. Therefore, we wanted to investigate the effect of an early phosphoantigen encounter on infant Vγ9Vδ2 T cells. For that, blood was collected from 10-week-old infants vaccinated at birth or not with the phosphoantigen-containing BCG vaccine as well as from newborns and adults. We performed flow cytometry assays ex vivo or after in vitro stimulation of PBMCs and analysis of the γδ TCR repertoire. Our data indicate that there is no significant difference on the phenotype or the effector functions and that there is no outstanding change in the γδ TCR repertoire between the vaccinated and non-vaccinated infants. However, infant Vγ9Vδ2 T cells, independent of their BCG status, showed striking differences compared to their neonatal counterparts such as a high expression of the cytotoxic mediator granzyme B and perforin, which was not observed in other γδ T cell subsets. In conclusion, our data indicate that other environmental encounters of infants are more important than BCG vaccination for the early phenotypic and functional evolution of the Vγ9Vδ2 T cells.

P.A5.05.12 Role of PARP-1 in regulatory Foxp3CD4+ T and helper-17 T cell differentiation
F. Novelli, C. Pioi
ENEA, Division of Health Protection Technologies, Rome, Italy.

Recent findings highlighted the role of ADP-ribosylating enzymes in inflammation and immune responses, with PARP-1 (polyADPriboselpolymerase-1) playing a relevant role in leukocyte activation and differentiation. We had found that PARP-1 deficient (PARP-1−−) mice display increased number of regulatory CD4 Foxp3+ T cells (Tregs) in central as well as peripheral lymphatic organs compared with wild-type (WT) controls. PARP-1−/− Tregs were functional as assessed both in vitro and in vivo. While in a chimeric competitive assay PARP-1KO thymocytes generated higher numbers of WT T cells, we wondered whether co-culture of naive CD4 cells to inducible Tregs (iTregs) was also affected. Purified naïve CD4 cells from PARP-1−/− mice, stimulated in vitro with CD3/CD28 and TGFβ1, expressed Foxp3 mRNA at higher levels and generated a greater number of Foxp3+ iTregs than the WT counterpart. Interestingly, in vitro differentiation of purified naïve CD4 cells to Th17 cells, as induced by CD3/CD28, TGFβ1 and IL-6, was not affected by PARP-1 deficiency. In peripheral lymphatic organs, conversion to Foxp3+ iTregs occurs upon stimulation by dendritic cells (DCs) in a tolerogenic context. Noteworthy, we found that purified WT naive CD4 cells, stimulated with CD3 and TGFβ1 in the presence of either WT or PARP1-KO DCs, generated comparable numbers of Foxp3+ iTregs. At variance, WT DCs induced a higher frequency of IL17+CD4+ cells compared with PARP1-KO DCs. Altogether, these results indicate that PARP-1 plays an important role in the balance between regulatory and Th17 cell differentiation with the involvement of both CD4 T cell intrinsic and DC-mediated effects.

P.A5.05.14 CD1b presents Borrelia burgdorferi glycolipid to human T cells
1Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands; 2Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital and Harvard Medical School, Boston, United States; 3Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia; 4Department of Medical Microbiology and Immunology, Diakonessenhuis Hospital, Utrecht, Netherlands; 5Laboratory for Infectious Diseases and laboratory Surveillance, Centre for Infectious Diseases Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands; 6National Institute of Child Health and Human Development, National Institute of Health, Bethesda, United States; 7Center for Immunology and Inflammatory Diseases,Massachusetts General Hospital, Boston, United States, 8ARC Centre of Excellence in Advanced Molecular Imaging, University of Melbourne, Melbourne, Australia.

Lyme disease is caused by the spirochete Borrelia burgdorferi. Two ubiquitous lipids of Borrelia burgdorferi, BBGl-I and BBGl-II, comprise 35% of the total lipid mass of the bacteria and are specifically synthesized by pathogenic Borrelia spp. It is known from previous studies that BBGl-II can bind to CD1d and activate CD1d restricted NKT cells. In this study, we carried out FACS-sorting with CD1b-BBGl-II tetramers, to obtain a T cell line from a Lyme disease patient that recognizes BBGl-II presented by CD1d. The T cell clone binds to CD1b-BBGl-II tetramers but not to mock loaded CD1b tetramers or CD1b loaded with negative control lipid phosphatidyglycerol. Although there is specific recognition of the CD1b-BBGl-II complex using tetramers, the primary T cells produce comparable levels of IFN-γ in an ELISPOT assay when stimulated with CD1b-expressing antigen presenting cells in the presence or absence of BBGl-II lipid. Whereas the primary T cells preferably bind to CD1b tetramers loaded with BBGl-II, we think that the activation by antigen presenting cells without the addition of BBGl-II is caused by a combination of a low affinity interaction with endogenous lipid loaded CD1d and a high expression level of CD1d on the antigen presenting cells. We call this phenomenon “antigen-modulated autoreactivity” against the CD1b molecule, where there is an increased reaction to an antigen over a baseline autoreactivity of the T cell towards CD1b. Furthermore, Lyme disease patients were screened for the presence and frequency of CD1b-BBGl-II specific T cells using CD1b tetramers.

P.A5.05.15 The role of the transcription factor Interferon-Regulatory Factor 4 in regulation of Th17 cells
C. Schmidt, A. Harterts, F. Raczkowski, H. W. Mittrucker
Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

The transcription factor Interferon Regulatory Factor 4 (IRF4) is an essential regulator of CD4+ T cell maturation to different Th cell lineages. As a consequence, IRF4 is essential for effective T cell responses against various antigens. We aim to characterize the role of IRF4 in Th17 cell differentiation, as well as in the maintenance of the differentiation status and function of these cells. CD4+ T cells from irf4−/−, irf4−/− and irf4−/− mice are stimulated under Th17-inducing conditions and cultured for up to three weeks with IL-7 and IL-23. At different time points, the expression of lineage-specific transcription factors and cytokines is determined by intracellular mAb staining and FACS. In addition, CD4+ T cells from irf4−/− x CreERT2 mice are stimulated and the remaining functional irf4 allele is subsequently deleted by CreERT2 activation with tamoxifen. First results indicated that induction of Th17 cells strictly depended on the presence of IRF4 during T cell activation, since irf4−/− T cells failed to upregulate RORγt and to produce IL-17A. In contrast, when the functional irf4 allele was deleted in CD4+ T cells from irf4−/− x CreERT2 mice after stimulation, cells retained the capacity to produce IL-17A. So far our results suggest that maintenance of Th17 cells is less dependent on IRF4 than the induction of these cells. Further studies are planned to investigate the role of IRF4 in induction and stability of Th17 cells in vivo in a mouse infection model.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 197
P.AS.05.16 Identification of factors driving induction of liver resident CD8+T cells following viral vector vaccination


CD8+ T cells play a pivotal role in mediating protection from liver-stage malaria, but this protection requires high numbers of CD8+ T cells which are able to locate and kill infected cells during the short time parasites are present in the liver. To improve viral vectored vaccine efficacy, we recently developed a two-step vaccination approach, termed “prime-target”, where CD8+ T cells are primed by intramuscular vaccination, followed by an intravenous administration of viral vector, enabling targeting of CD8+ T cells to the liver. This approach leads to high numbers of CD8+ T cells in the liver and much greater levels of efficacy against malaria sporozoite challenge. While both effector and tissue resident (TNR) cells alone can mediate protection, TNR cells are more efficient; we therefore aimed to identify the optimal vaccination regimen for induction of TNR cells.

In this study we tested three different viral vectors, Adenovirus, Modified Vaccinia Ankara and Adeno-Associated Virus, for their abilities to target CD8+ T cells to the liver. In response to all vectors, we observed a negative correlation between the frequency of antigen specific CD8+ cells and frequency of TNR cells, however the ratio differed between vectors. Each vector was evaluated in the level and duration of antigen expression and induction of pro-inflammatory signals, suggesting that both of these factors could have an impact on TNR induction. Further experiments are therefore underway to elucidate the impact of antigen level and inflammatory signals on the induction of TNR cells.

P.AS.05.17 Human liver- and skin-derived NK cells exhibit antigen-specific memory responses

V. Stary, J. Ströbl, P. Starlinger, G. Stary; Medical University of Vienna, Vienna, Austria, Vienna, Austria.

Mounting evidence suggests that NK cells can develop long-lived and highly specific memory to a variety of haptens and viral antigens in mice and in non-human primates. The existence and consequences of antigen-specific NK cell memory still needs to be proven. We isolated NK cells of human livers and blood from individuals vaccinated against hepatitis A and/or B, characterized them phenotypically and functionally in killing assays against antigens the patients had been vaccinated. We evaluated the distribution and function of NK cells in epithocytic patch test reactions of nickel-sensitized patients, an effector site of adaptive immune responses. In contrast to the peripheral blood, two distinct NK cell populations were found in the liver based on their expression of CD16 and CD49a. CD49a+CD16+ liver NK cells (54.6% ± 4.2 of total NK cells) performed antigen-specific killing of hepatics A or B-pulsed autologous B cells matching the patients’ vaccination status. Blood-derived and CD49a+CD16+ liver NK cells did not exhibit antigen-specific cytotoxicity, but recognized MHC-I+ target cells. MHC-I+NK cell-redirected NK cell subsets were capable of specific lysis of nickel-pulsed autologous target cells. These results suggest that antigen-specific memory NK cells in humans are present in the liver and, in contrast to adaptive immune responses, as effector cells in influenza skin. The underlying mechanisms for specific recognition of viral antigens and haptens by human memory NK cells might form the basis to target NK cells.

P.AS.05.18 The soluble cytoplastmic tail of CD45 (cd-CD45) in human plasma contributes to keep T cells in a quiescent state

A. Puck1, S. Hofg2, M. Modak1, O. Maij1, P. Cejka1, S. Blüm1, C. Arnold-Schraud2, J. G. Sierwen3, K. S. Frederiksen1, E. Theil1, J. Leitner1, P. Steinberger4, R. Aigner1, M. Seyerl-Jireš6, G. J. Zilbing1, J. Stöck1.

1Institute of Immunology, Medical University of Vienna, Vienna, Austria, 2Department for Rheumatology, Medical University of Vienna, Vienna, Austria, 3Novo Nordisk A/S, Biopharmaceuticals Research Unit, Måløv, Denmark, 4Department for Gynecology, St. Josef Hospital, Vienna, Austria.

The cytoplasmic tail of CD45 (cd-CD45) is proteolytically cleaved and released upon activation of human phagocytes. It acts on T cells as an inhibitor, cytokine-like factor in vitro. Here we show, that cd-CD45 is abundant in human peripheral blood plasma from healthy adults compared with plasma derived from umbilical cord blood and plasma from patients with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). Plasma depleted of cd-CD45 enhanced T cell proliferation, while addition of exogenous cd-CD45 protein inhibited proliferation and reduced cytokine production of human T lymphocytes in response to TCR signaling. Inhibition of T cell proliferation by cd-CD45 was overcome by co-stimulation via CD28. T cell activation in the presence of cd-CD45 was associated with an upregulation of the quiescence factors Schlafen family member 12 (SLFN12) and Krueppel-like factor 2 (KLF2) as well as of the cyclin-dependent kinase (CDK) inhibitor p27kip1. In contrast, positive regulators of the cell cycle such as cyclin D2 and D3 as well as CDK2 and CDK4 were found to be downregulated in response to cd-CD45. In summary, we demonstrate that cd-CD45 is present in human plasma and sets the threshold of T cell activation.

P.AS.05.19 Resting memory CD4 T cells are generated following prolonged cell division

J. Sarkander, M. Mursell, Y. Yamasaki, S. Hoya, K. Tokoyoda; Deutsches Rheuma-Forschungszentrum Berlin, Berlin, Germany.

CD4 T cell memory is fundamental for long-lasting immunity and effective recall responses following infection or vaccination. We have so far determined that resting memory CD4 T cells specific for systemic antigens preferentially reside in the bone marrow (BM) and that splenic CD49b+/CD16+ memory CD4 T cells are the precursors of BM memory precursors. Following the sufficient cell division, memory precursors can specifically downregulate CCR7 and upregulate IL-2Rβ, suggesting that loss of CCR7 and gain of IL-2 signaling are required for the migration and survival of the precursors of BM memory CD4 T cells, respectively.

P.AS.05.20 Initiation of immune responses - Part 6

V. Stary, J. Ströbl, P. Starlinger, G. Stary; Medical University of Vienna, Vienna, Austria, Vienna, Austria.

New insights into mechanisms of sterile inflammation

N. Freise, A. Burghard, T. Ortkras, N. Daber, T. Vogl, J. Roth, J. Austermann; Institute of Immunology, Muenster, Germany.

Background: Septis is a disease, caused by pathogens, that is still associated with high mortality rates worldwide. After an early strong inflammatory phase, sepsis patients might develop into a more hypo-responsive state, called endotoxin-tolerance. In this case, invading pathogens cannot be recognized by the immune system and secondary infections can arise. However, in 30% of sepsis patients an initial microbial trigger is missing. We recently demonstrated that under sterile conditions endogenous proteins like the alarmins Stimulator of Interleukin-1 Receptor (STING) and TLR7/8 are able to induce a hypo-responsiveness of phagocytes, a mechanism we called stress-tolerance.

Objective: The goal of the present study was to analyze molecular mechanisms underlying stress-induced tolerance in phagocytes and their relevance in vivo.

Methods: We investigated the activation of certain signaling pathways in stress-tolerant human or murine phagocytes and their relevance in vivo. Therefore we performed ImageStream, multiplex ELISA and western blot analysis and a D-Gal model of septic shock. We also analyzed blood samples of cardiology bypass patients.

Results: We identified two main signaling pathways to be involved in STING-induced tolerance of phagocytes: the PI3K/AKT/GSK3 and the JAK/STAT pathway. In vivo data show a protective effect of a GSK3 inhibitor on the survival of mice during septic shock. Furthermore, master regulator proteins of the JAK/STAT pathway seem to have an important role in phagocytes of cardiopulmonary bypass patients.

Conclusion: The alarmins STING and TLR7/8 induce stress-tolerance in phagocytes via the PI3K/AKT/GSK3 and the JAK/STAT pathway, relevant for development of a hypo-responsive state in hypoinflammation in cardiology bypass patients.

P.AS.05.21 Vitamin B complex therapy suppresses neuroinflammation and improves recovery of injured peripheral motor nerve

B. Bozic Nedeljkovic, S. Dacic1, P. Nedeljkovic1, A. Ehmedah3, B. Draskovic Pavlovic1, D. Vucevic1, S. Pekovic1,4.

1Faculty of Biology, Belgrade, Serbia, 2Institute for Orthopedic Surgery “Banjica”, Belgrade, Serbia, 3Military Medical Academy, University of Defense in Belgrade, Belgrade, Serbia, 4Institute for Biological Research “Sinisa Stankovic”, Belgrade, Serbia.

Statement of the Problem: Peripheral nerve injury (PNI) leads to series of cellular and molecular events necessary for axon regeneration and reinnervation of target tissues. Macrophage recruitment that occurs immediately after PNI aids production of cytokines and neurotrophic factors necessary for axon regeneration. Calcium entry via L type of voltage-dependent calcium channels (LVDCCs) is involved in the processes underlying macrophage activation. The aim of this study was to evaluate influence of vitamin B complex therapy on: recovery of motor function after PNI; processes of neuroinflammation that are in part regulated by Ca2+ subunits of LVDCCs. Methodology: Adult male rats were used. Surgery: Motor branch of femoral nerve was transected and reconstructed by end-to-end anastomosis. Experimental groups: (O) operated, (OT) operated and daily treated with vitamin B complex for 14 days. (S) sham-operated animals, underwent the same procedure but without transection of nerve. Pre- and post-operatively behavior tests were performed. Animals were sacrificed 1, 3, 7, and 14 days post-injury.
POSTER PRESENTATIONS

Findings: Treatment with vitamin B complex applied immediately after PNI enhanced recovery of walking function, decreased muscle atrophy and improved musculature quality. Pro-inflammatory activity evaluated by EMG. Additionally, it decreased pro-inflammatory and increased anti-inflammatory cytokines expression and reduced Ca^2+ LVDCC's activation in macrophages. Conclusion & Significance: Vitamin B complex therapy down-regulates expression of Ca^2+ LVDCCs on activated macrophages and suppresses neuroinflammation, thereby contributing to motor function recovery of injured nerve, suggesting possible implementation in therapy of PNI, which remains to be explored. Acknowledgement: Supported by grants IH40104, 175033, MFVMA/10/16-18

P.A5.06.03

TLR antagonist immunosuppressive A151 ODN acts through metabolic reprogramming by suppressing PI3K/AKT/mTOR pathway

O. Bulut1, V. Vázquez2, G. Kilic1, N. Arsenovic-Ranin3, M. G. Leposavic2, I. Gursel1, B. Jenewein1, B. Grubeck-Loebenstein1

1Institute of Virology, Vaccines and Sera "Tovork", Belgrade, Serbia, 2University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia, 3University of Belgrade-Faculty of Medicine and Life Sciences, Tampere, Finland

199

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
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P.A5.06.09
Human bone marrow macrophages display distinct immune regulatory properties
C. Miglitusch1, B. Jenewein1, A. Meryk1, K. Triebl1, W. Zwerschke1, B. Grubeck-Loebenstein1
1Institute for Biomedical Aging Research, Innsbruck, Austria, 2Department of Orthopedic Surgery, Klinikum Wels, Wels, Austria.

The bone marrow (BM) is a primary lymphoid organ of the human immune system where T and B cell precursors are generated and antigen-experienced adaptive cells are maintained. The BM has proven to be a major reservoir of resting memory T cells and long-lived plasma cells, capable of providing protection against recurrent infections. The survival and maintenance of these cells is mediated by cytokine and chemokine producing stromal cells and myeloid cell types, forming specific areas known as BM niches. However, BM is also available on the production of myeloid cell types. In T cell survival factors by BM fat tissue and the interaction with adaptive immune cells in the BM. Using microarrays, we show that bone marrow fat significantly differs from subcutaneous fat regarding specific gene expression profiles including inflammatory responses. Reduced expression levels of the adipocyte-specific genes may suggest that the BM is an immune regulatory organ. Higher expression of the effector/memory T cell survival factors IL-7 and IL-15 were found in BM compared to subcutaneous adipocytes. The expression of the pro-inflammatory molecules TNFα and IL-6, which contribute to the low-grade inflammatory bystander effect, known as “inflammaging” observed in elderly persons, was also higher in BM fat. With our data, we can show that the unique phenotype of BM adipocytes expressing pro-inflammatory cytokines may have a negative effect on long-lived plasma cells while maintaining effector/memory T cells.

P.A5.06.10
The role of thio-reiodoxin interacting proteins (TXNIP) in T cell activation
S. Nagel1, S. Ziola1, P. Kramer1, K. Gülow1

1German Cancer Research Center (DKFZ), Heidelberg, Germany, 2University Hospital Regensburg (UKR), Regensburg, Germany.

T cells undergo rapid proliferation and differentiation upon stimulation of the T cell receptor (TCR). We have shown that regulation of TCR-induced oxidative signaling is crucial for control of a T cell immune response. TXNIP is a negative regulator of the oxidative defense controlling the intracellular redox equilibrium. Thus, TXNIP is a promising candidate for regulating T cell signaling. We demonstrate that TXNIP is downregulated independently of ROS upon TCR activation. To examine the molecular mechanism and the resulting effects of TXNIP downregulation upon TCR triggering a KRASp-Ca²⁺ knockout of TXNIP (TXNIP KO) in Jurkat T cells was generated. By means of TXNIP KO clones the role of TXNIP in T cell activation was addressed. Thereby, we have shown that TXNIP deficiency has no impact on activation-induced oxidative signaling. Nonetheless, we could determine that TXNIP KO T cells show enhanced CD95 death ligand (CD95L/FAS/APO-1-L) expression as well as activation-induced cell death (AICD) upon TCR triggering. Therefore, TXNIP seems to have an important role in T cell activation and co-stimulation wherein it regulates several aspects of T cell fate decisions.

P.A5.06.12
Identification of human self-reactive INKT cells
J. PERROTEAU1, L. Hesnard2, M. Devidel1, B. Noveli1, L. Gapon1, E. Scocelli1, L. Gautreau-Rolland1, X. Soulquin1, 1Centre de Cancérologie et Immunologie de Nantes, Nantes, France, 2National Jewish Health - University of Colorado, Denver, United States.

Invariant Natural Killer T (iNKT) lymphocytes express both NK receptors and a semi-invariant αβ TCR restricted by the CD1d molecule presenting glycolipids. Among them, αGalactosylCeramide (αGC) is a potent ligand of all iNKT cells. In some contexts, iNKT cells are also able to detect endogenous glycolipids, which highlights their self-reactivity. However, the mechanisms underlying this autoreactivity are still poorly understood.

By using a tetramer-associated magnetic approach, we generated several INKT cell lines from the peripheral blood of healthy donors. These cells reacted similarly against high ICAM-1 target cells after loading with αGC, but in a different manner against unloaded TR164- target cells, expressing endogenous glycolipids, both in terms of cytotoxicity and cytokines production. Moreover we demonstrated that the autoreactivity is dependent on TCR-CD1d signaling. The analysis at the clonal level (n=12) of an autoimmune cell line also revealed an important heterogeneity between clones in terms of self-reactivity. We found identical alpha and beta chain TCR sequences in all the clones obtained, suggesting that expression of particular TCR sequence is not sufficient to induce self-reactive. Comparative RNAseq analysis revealed a direct correlation between the tyrosine kinase SYC expression level and autoreactivity, while an inverse correlation was observed with the expression of the phosphatase DUSP2. As SYC and DUSP2 are respectively implicated in TCR signal transduction and modulation of Th17 cells development (Muro et al., 2018; Lu et al., 2015), our results suggest that the balance of expression of these 2 proteins could modulate the intrinsic autoreactive potential of human INKT cells.

P.A5.06.13
Neutrophils driving unconventional T cells are essential for resistance to sarcomas
A. Ponzeiro1, M. Barbagallo1, R. Carrierio1, M. Molgora2, C. Perucchini1, S. Carnevale1, E. Magrini1, F. K. Riederer3, F. Pirolt1, S. Di Marco1, D. Supino1, S. Pilotti1, E. Bonavita1, M. Galdiero3, C. Garlando1, A. Mantovani1, S. Jallon1
1Humanitas Clinical and Research Center, Rozzano, Italy, 2Fondazione IRCCS Istituto Nazionale Tumor, Milano, Italy, 3Humanitas University, Pieve Emanuele, Italy.

Neutrophils represent a fundamental mechanism of antimicrobial resistance and inflammation. Moreover, neutrophils have emerged as important players in the activation, orchestration and regulation of adaptive immune responses. Neutrophils are a component of the tumor microenvironment (TME) and have been shown to promote progression. On the other hand, unleashed neutrophilic effectors have also been reported to mediate anti-cancer resistance. Antibody-mediated depletion used to investigate the role of neutrophils in tumor progression suffers from limitations, including duration, specificity and perturbation of the system. We therefore used a genetic approach to investigate the role of neutrophils in mouse models of sarcoma and lymphoma in order to provide insight into the mechanisms of neutrophil-mediated anti-tumor responses and antitumor immunity.

P.A5.06.14
Investigating the molecular basis of Roquin-mediated control of T cell fate decisions
H. Schmidt1, V. Heissmeyer1
1Ludwig-Maximilians-Universität (LMU), Biomedical Center (BMC), Institute of Immunology, Planegg-Martinsried, Germany, 2Helmholtz Zentrum, Munich, Germany.

Post-transcriptional gene regulation by RNA-binding proteins (RBPs) controls T cell fate decisions. The RBPs Roquin-1 and -2, encoded by the genes Rch3 and Rch2, serve redundant functions in T cells. They bind via their ROQ domain to 3'UTRs of mRNAs and control mRNA stability and expression. Interestingly, someonque mutant mice harboring a single ROQ domain target mutation or mice lacking Roquin-1/2 protein expression revealed accumulations of Th1 and Th17 or Th1 and Th17 effector T cells, respectively. Since the Roquin-1β gene is a functional hypomorph of Roquin-1, we hypothesize that the molecular regulation of Th1 and Th17 differentiation is determined by a graded loss-of-function of Roquin. Therefore, we investigate the effects of (i) graded Roquin reduction by increasing deletion of Rch3 and Rch2 alleles, by (ii) introducing ROQ domain mutations that attenuate affinities to mRNAs and (iii) pharmacologically modulating MAAT1-induced cleavage of Roquin or stimulating T cells with increased TCR signal strength. Our preliminary data indeed indicate that graded loss of Roquin correlates with increased effector-memory phenotypes of CD4 and CD8 T cells and ex vivo analyses suggest graded target upregulation including ICOS, CXCL10 and CTLA-4 transcripts. Furthermore, minimal amounts were still effective to suppress Th17 differentiation as measured by IL-17A and RORγt expression. In future studies, we will seek to uncover how graded T cell stimulation regulates differential Roquin activities that mediate T lymphocyte fate-specifying mRNA repression.

P.A5.06.15
Noradrenaline synthesized locally in draining lymph node modulates CD4+ T cell development in rat EAE model: a role for α, adrenoceptor
I. Pilipovic1, I. Vujnovic1, R. Petrovic1, D. Kosec2, Z. Stojic-Vukunic3, G. Lesapic2
1Institute of Virology, Vaccines and Sera “Torlak”, Belgrade, Serbia, 2University of Belgrade - Faculty of Pharmacy, Belgrade, Serbia.

Introduction: It has been suggested that “noradrenaline in “adrenergic” immune cells changes development of EAE and multiple sclerosis and noradrenaline influences EAE development through α, adrenoceptor. To elucidate mechanisms standing behind this phenomenon, α1, adrenoceptor-mediated influence of draning lymph node (DLN) cells derived noradrenaline on CD4+ T cell response in DLN from Dark Agout rats of both sexes immunized for EAE was examined. Methods: Cells recovered from DLN on 7th day post-immunization were examined for noradrenaline synthesis/content and α1, adrenoceptor expression using HPLC and/or flow cytometry. Additionally, effects of prazosin (α1B, AR blocker) on CD4+ T cell proliferation, the frequency of IL-17+ CD4+ T cells and regulatory (Foxp3+CD25+) CD4+ T cells (Tregs), activation/maturational/maturational state on antigen presenting cells (APCs) and their cytokine profile in DLN cell culture examined. Experiments were performed in cell culture and/or for co-culture. Results: Ine of EAE, conventional CD4+ T cells, Tregs, and APCs from rats no DLN synthesized noradrenaline, while only Tregs and APCs expressed α1, adrenoceptor. In myelin basic protein-stimulated DLN cell cultures from rats of both sexes prazosin increased Treg frequency and Foxp3 expression, but diminished co-stimulatory CD80 and CD86 molecule expression on APCs, thereby reducing CD4+ T cell proliferation.
Additionally, prazosin diminished expression of TH17 polarizing cytokines (IL-1β and IL-23) in dLN cell cultures, and reduced the frequency of all IL-17+ CD4+ T-cells, and those coexpressing GM-CSF. The study indicates that in rats of both sexes immunized for EAE, dLN cell-derived noradrenaline through α₂-adrenoceptor influence generation of (auto)immune IL-17+ CD4+ T-cell response and thereby EAE development. (Grant 175050, MESTD, Republic Serbia).

P.A.S.06.16
Increased expression of checkpoint inhibitors on CD4+ T-cells during CPS immunisation is associated with slower acquisition of immunity
X. Yap1, J. Walki1, J. J. Reuling2, W. Gramans2, G. van Gemert3, R. Siebelink-Stoter2, M. van de Vege-Bolmer1, K. Keelen1, E. M. Bijker1,4, A. Scholten1,5, R. W. Sauerwein1;
1Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands, 2Department of Pediatrics, Radboud University Medical Center, Nijmegen, Netherlands, 3Innotas Laboratories B.V., Os, Netherlands.

Malaria poses a significant burden to global health, yet much remains unknown about the development of immunity to the malaria parasite Plasmadium falciparum. Chloroquine chemoprophylaxis with P. falciparum sporozoites (CPS) immunisation induces highly effective sterile protection in naive volunteers. However, some volunteers are protected after the first immunisation (fast responders), whereas others require two or more immunisations to be fully protected (slow responders). Checkpoint inhibitors are regulatory molecules which inhibit immune responses, including acquisition of adaptive immunity. Blocking checkpoint inhibitors drastically improves survival in murine malaria studies. However, the importance of checkpoint inhibitors in malaria vaccination has not yet been established.

Expression of checkpoint inhibitors CTLA4, TIM3, and PD-1 was measured by flow cytometry on CD4+, CD8+, and γδ T-cells, and NK cells from 32 immunised volunteers in two CPS immunisation trials (NCT02080026, NCT02098590). Expression of TIM3 on CD4+ and CD8+ T-cells (p=0.0167, p=0.0152) and CTLA4 on CD8+ T-cells (p=0.0255) differed significantly between fast and slow responders. Furthermore, when expression of all antibody markers was summed up into a cumulative inhibitory Z-score, CD4+ T-cells from fast responders had a significantly lower inhibitory score (p=0.0234) compared to slow responders.

This study demonstrates for the first time that fast responders to malaria vaccination have lower checkpoint inhibitor expression than slow responders. Further studies will examine whether individuals vary in their capacity to express checkpoint inhibitors and produce immunosuppressive cytokines after restimulation with P. falciparum-infected erythrocytes. These findings provide insight into how individual immunosuppressive profiles prior to vaccination can affect malaria vaccination efficacy.

P.A.S.06.17
CHARACTERIZATION OF HUMAN MONOCLONAL ANTIBODIES AGAINST DENGUE VIRUS NS1 PROTEIN
K. Sripaire, Panannthip Pitaksajakul, Pongrama Ramosoto, Khwanich Boonha, Wilrat Porungmanee;
Tropical Medicine, Bangkok, Thailand.

Background: Dengue hemorrhagic fever caused by dengue viruses is a public health problem in tropical and subtropical regions. Today, dengue is considered one of the most important arthropod-borne viral diseases in humans in terms of morbidity and mortality. The dengue virus (DENV) non-structural 1 (NS1) protein plays a critical role in viral RNA replication and has a central position in DENV pathogenesis. In the last three decades, the DENV NS1 protein has also been intensively investigated as a potential target for vaccines and immunotherapy. However, anti-NS1 antibody was recently interested as one factor of severe dengue infection due to their cross-reactivity with human molecules such as endothelial cell, integin and plasminogen, causing some severe symptoms like vascular leakage. Project description: To identify anti-NS1 human monoclonal antibodies (HuMabs), in this study, HuMabs were generated by hybridoma technology by fusing of human PBMCs with human fusion partner cell (SP2/MEG). HuMabs specific for NS1 protein of dengue virus were screened and confirmed by western blot analysis. Target epitope of anti-NS1 was also determined by random peptide phage display. Genetic information of those anti-NS1 HuMabs was elucidated. Conclusion: This is the first study described the generation and characterization of full IgG human monoclonal antibody specific to NS1 protein of Dengue virus. These characterizations could be used for a study of DENV pathogenesis and dengue vaccine candidates in the future.

P.A.S.06.18
Human oral epithelial cells inhibit Th1 cell responses in a cell contact-dependent manner
Department of Immunology, School of Medicine, Madrid, Spain.

The oral mucosa is a site of intense immunological activity, where tolerogenic and defensive responses are articulated. The underlying mechanisms resulting in active immunity or tolerance are poorly understood but it is evident that oral epithelial cells (OECs) of the mucosa ought to play an important role. Here, we characterized the ability of human oral squamous cell carcinoma cell lines and primary oral epithelial cells to modulate immune responses. OECs constitutively express CD40 and respond to inflammatory stimulation with increased IL-12 production without CD80 and CD86 costimulation. Co-culture of OECs with dendritic cells (DCs) drastically reduced IL-12 released by DCs after exposure to bacteria and induced a tolerogenic phenotype characterized by reduced MHC II, CD80 and CD86 expression and increased IL-10 production in the presence of primary OECs. OEC-conditioned DCs were unable to promote Th1 differentiation as determined by a lack of IFNγ production in allogenic activated CD4+ T-cells. Moreover, OECs were able to abrogate CD25 and CD69 expression, T cell proliferation and the release of IFNγ and TNFα when co-cultured with anti-CD3+anti-CD28 stimulated CD4+ T-cell. The inhibition of T cell activation was TGF-β independent but cell-contact dependent. Our data indicate that the oral epithelium promotes an anti-inflammatory state by conditioning DCs maturation and function.

P.A.S.06.19
Phenotype of monocyte-derived dendritic cells in response to halophilic archaea Halorhabdus rudnickiae and Natrinema salaciae
K. Krawczyk1, A. Biekier2, M. S. da Costa1, J. M. Albuquerque3, M. Kowalewicz-Kulbat1;
1Department of Infectious and Environmental Medicine, Faculty of Veterinary Medicine, Faculty of Biology, University of Coimbra, Coimbra, Portugal.

Halophilic archaea are one of the three domains of life. This domain comprises many extreme halophiles, defined as microorganisms that inhabit hypersaline environments. Halophilic archaea strains of Halorhabdus rudnickiae WSM-64 and WSM-66, were isolated from the hypersaline environment in Barycz mining area belonging to the Polish Salt Mine Company “Wieliczka”, Natrinema salaciae strain MDB25 was isolated from the deep, hypersaline anoxic Lake Medee in the Eastern Mediterranean Sea. The role of archaea as part of the human microbiome has been described but still remains unknown how halophiles can interact with the human cells.

Monocytes to monocyte-derived dendritic cells (Mo-DC) and dendritic cells (DC) on the phenotype of the human monocyte-derived dendritic cells (Mo-DC).

Conclusion: Our results suggest that halophiles possess the ability to diminish some signals in oral mucosa which may have an impact on the naive T cell differentiation. Further research should provide insights into DC and T cell cytokine production.
POSTER PRESENTATIONS

P.AS.07.02

CDSignalosome coordinates TCR signals to control the generation of peripherally induced regulatory T cells

G. BLAIZE1, N. Rouquié2, M. Marcellin3, M. Benamar4, A. Gonzales de Peredo5, O. Schiltz6, R. Lesseronne1

1CPTP, INSERM U1043, CNRS, UMR 2822, Toulouse, France; 2I3B, UMR 5609, Toulouse, France.

Introduction: CD5 proteins are TCR co-receptors initially described as negative regulators of T cell signaling and T cell responses. Despite many studies performed mainly in cell lines, the molecular mechanisms mediated by CD5 on primary T cells remain unclear.

Results: We performed mass spectrometry (MS) analysis of CD5 partners in primary T cells. We identified a molecular complex recruited on CD5 upon TCR engagement. In contrast to the presumed classical role of CD5 as a co-repressor, our results suggest that CD5 is involved in the recruitment of a set of proteins involved in the regulation of T cell responses.

Concluding: In this work, we revisited mechanisms and functions by identifying Y429 of CD5 as a critical residue to recruit CD5 partners. Our work suggests that CD5 could optimize immune responses by setting the threshold for conventional T cell and p70 biological functions.

P.AS.07.04

Investigating the interaction and orientation of the immune cell proteins CD2, CD4 and CD45 on model membranes using hydrodynamic trapping

V. Junghans1, A. M. Santos1, S. J. Davis1, P. Jönnson2

1Lund University, Lund, Sweden; 2University of Oxford, Oxford, United Kingdom.

Different proteins play an important role during the cell-cell contact formation and are highly organized in respect to their size and function. However, crucial information about intermolecular interactions and height-dependent orientation of the proteins, especially the glycoprotein CD45, are lacking. We show by using hydrodynamic trapping (HDT) how these missing parameters for the immune-cell molecules CD2, CD4 and CD45 can be obtained.

In HDT a micropipette is positioned above a supported lipid bilayer (SLB) and negative pressure applied through the micropipette results in accumulation of the proteins attached to the SLB1. Relating the protein accumulation to the trapping strength both the molecular size/orientation as well as the intermolecular force between the proteins can be determined. In our system CD2 and CD4 oriented in an upright position from the bilayer, whereas CD45 had more freedom rotating relative to the surface. With increasing surface coverage, this flexibility reduced and CD45 positioned at the surface at lower coverage. Our results demonstrate the potential of HDT for the determination of intermolecular forces.

P.AS.07.05

Nanobodies from transgenic mice


University Medical Center Hamburg, Hamburg, Germany.

Llamas and other camelids carry a variant immunoglobulin locus that encodes antibodies composed only of heavy chains. The single variable domain of these antibodies (designated VH or nanobody) has been shown to have high solubility and stability, independent of a partner VL domain. With their long CD3Rs, nanobodies can reach hidden epitopes that are not accessible for conventional antibodies. Nanobodies can be used in monovalent format, e.g. for high resolution microscopy or as crystallization chaperones. Due to their high solubility and stability nanobodies can readily be fused to other proteins. For example, fusion to other nanobodies yields bispecific or multispecific reagents, fusion to the hinge and Fc domains heavy chain antibodies of any desired isotype. In order to facilitate the generation of nanobodies for biomedical applications, we have developed a platform for generating nanobodies by transgenic engineering of an llama IgH locus to IgH-kO mice. Immunization of these mice induces antigen-specific heavy chain antibody responses with efficient VDJ recombination, somatic hypermutation, and class switch from IgM to IgG. These mice thus provide a flexible new platform for generating innovative nanobody-based biologics. They also provide a basis for genetic modification of nanobodies and the generation of designer nanobodies.

P.AS.07.06

MHC presentation is limited by the availability of MHC molecules rather than by the supply of peptide ligands

L. R. Komov, D. Melamed Kadosh, E. Barnea, A. Admon

Technion-Israel Institute of Technology, Haifa, Israel.

Despite the importance of peptide presentation by the MHC class I, some aspects of the MHC processing and presentation are yet unexplored. One of those questions is whether the MHC presentation level is limited by the availability of peptide ligands within the ER or by the supply of peptide-receptive (empty) MHC molecules. Our study clarifies this issue by inducing major perturbations and competition for MHC ligands in human breast cancer cells (MCF-7). The cells were treated with interferons, which led to elevated presentation levels of the MHC-B molecules with their bound peptides, relative to the MHC-A and MHC-C molecules of the same cells. This result was unexpected, since all of the MHC alleles are present in the TRA-1-60 T cell line present on the same molecular level. These results were expected to have crucial effects on all of the MHC alleles. Furthermore, high expression levels of recombinant soluble MHC-A were induced in the cells, to create a competition for peptides between the soluble and the identical endogenous membranal MHC-A. This competition did not affect the membranal MHC-A presentation levels or its bound peptides. Our results suggest that in contrary to the common opinion, the MHC presentation levels are limited by the availability of peptide-receptive molecules rather than by the supply of peptides. These findings are important for the basic understanding of the antigen processing and presentation pathway, as well as vaccines design for pathogens infections and cancer immunotherapy. Supporting: the I-CORE Program of the Planning and Budgeting Committee and the Israel Science Foundation.

P.AS.07.07

Specialized pro-resolving mediators as novel therapeutic agents in treating neuroinflammation

A. Leut1, E. Biscioni1, A. Cordella1, V. Sasso1, M. D’Amelio1, M. Viscovì1, V. Churiù2,3

1Lipid Neurochemistry Unit, European Center for Brain Research (CERC), IRCCS Santa Lucia Foundation, Roma, Italy; 2Department of Medicine, Campus Bio-Medico University of Rome, Rome, Italy; 3Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy.

Specialized pro-resolving mediators (SPMs) are a novel class of endogenous lipids, produced by innate immune cells from essential omega-3 polyunsaturated fatty acids, that involve the antigen processing and presentation pathway, as well as vaccines design for pathogens infections and cancer immunotherapy. Supporting: the I-CORE Program of the Planning and Budgeting Committee and the Israel Science Foundation.

P.A.S.07.08

Inhibition of TLR and TLR+BCR dependent functions of human B cells by Complement Receptor Type 1 (CD35)

B. Mącis-Valent

Department of Immunology, Budapest, Hungary.

Although it is well accepted that separate activation of the complement system and Toll-like receptors (TLRs) initiates and shapes the adaptive immune response, much less is known about the modulation of various B cell functions by the simultaneous activation of these two systems. Therefore we investigated how engagement of complement receptor type 1 (CR1) influences the activation of human B cells induced by TLR7 and TLR9 with or without a BCR stimulus. Resting tonsilar B cells were activated via BCR by a suboptimal dose of F(ab’)2, anti-human IgM/IgA and via TLR7 and TLR9 by synthetic stimulators. The stimuli were applied either separately or simultaneously in the presence or absence of the CR1 ligand, a multicentric “C3b-like C3”. The effect of CR1 clustering was assessed on proliferation (“H-thymidine incorporation”), cytokine secretion (ELISA), antibody production (ELISPOT) and expression of activation markers (flow cytometry).
We show that CR1 clustering significantly and dose dependently reduces the TLR9-induced activation of tonsillar B cells, but has no effect on the TLR7-induced functions. The enhanced response to the simultaneous engagement of TLR7 or TLR9 with the BCR was also significantly correlated with CR1 clustering. Our data demonstrate that engagement of CR1 downregulates the TLR9-induced B cell functions but does not influence the TLR7 mediated processes. Interestingly however, when B cells are simultaneously triggered via BCR+TLR7 or BCR+TLR9, CR1 clustering inhibits the B cell response. We assume that CR1 exerts its inhibitory effect by acting on signalling molecules linked to both BCR and TLR9 in human B cells.

P.A.S.07.09

c-Myc in T lymphocytes: How is it controlled? What does it control?

J. M. Marchingo, L. V. Sinclair, D. A. Cannell; Cell Biology and Immunology Division, School of Life Sciences, The University of Dundee, Dundee, United Kingdom.

T cells undergo massive cell growth, rapid proliferation and differentiation to form a protective immune response. The proto-oncogenic transcription factor c-Myc plays a critical role in this process. To explore c-Myc function in T cells we used high-resolution mass spectrometry to compare the effect of c-Myc deficiency on the global proteome of antigen receptor-stimulated CD4 and CD8 T cells. Using this technology we quantified the expression of ~6700 proteins. c-Myc-deficiency reduced the copy number of ~4,400 and 2,400 proteins in CD8 and CD4 T cells respectively. The magnitude of c-Myc effect on individual proteins was selective. For example, while the amino acid transporter Slc7a5 was decreased 40 and 30-fold in CD4 and CD8 T cells, the glucose transporter Glut1 was 2.1 and 2.9-fold higher in the c-Myc-deficient CD4 and CD8 T cells respectively. In contrast Glut1 was the same across all conditions.

Systems with substantial biosynthetic defects can confound our ability to distinguish which proteins become dysregulated first and whether the effects are direct. For example Slc7a5 and c-Myc are both reported to regulate the other’s expression in T cells but deficiency in either also substantially compromises RNA and protein biosynthesis. We investigated how the reduced expression and translation levels interplayed with c-Myc expression. We found that c-Myc induction required extrinsic amino acids, but that Slc7a5 deficiency did not alter c-Myc induction; however it did compromise maintenance of c-Myc levels. Thus, c-Myc initiation of Slc7a5 triggers a positive feedback loop to maintain expression of both proteins and drive T cell growth.

P.A.S.07.10

sFasL mediates proinflammatory activation of neutrophils from type 2 diabetes mellitus without affecting apoptosis

S. Margaryan1,2, A. Wilkićowicz, A. Martirosyan; 1 Russian-Armenian University, Yerevan, Armenia, 2 Institute of Molecular Biology NAS RA, Yerevan, Armenia; 3 L. Hirsfeld Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

Introduction: Type 2 diabetes mellitus (T2DM) is a chronic metabolic condition characterized by insulin resistance and pancreatic β-cell dysfunction. Immune cell activation associated with persistent low grade inflammation play a prominent role in apoptosis-mediated β-cell destruction and vascular injury in T2DM. Despite Fas/sFasL axis was implicated in the development of diabetes-related complications, the role of soluble FasL (sFasL) is still unknown. Therefore, we aimed to analyze the influence of sFasL on neutrophil activation and apoptosis in vitro. Methods: For this, transcriptional and expression levels of pro-inflammatory and apoptotic genes were measured in neutrophils from T2DM patients (n=16) and healthy controls (n=15) exposed with sFasL for 3 hours. Results: sFasL significantly increased mRNA levels of NF-κB, IL-1β, caspase-3 in neutrophils isolated from T2DM patients which was associated with the increased CD18 MI. At the same time, apoptotic rates of the cells from both studied groups were unaffected by sFasL, which was accompanied by the unchanged mRNA levels of Ilx, decreased mRNA levels of caspase-3 and decreased number of Fas (CD95) positive neutrophils. In T2DM, the presence of sFasL resulted in increased production of IL-8 by whole blood cells compared to both control cultivation and sFasL-induced healthy cells. Conclusion: Thus, we showed an enhanced inflammatory response of neutrophils from T2DM patients to sFasL without acceleration of apoptosis, which may play an important role in induction and/or sustaining of inflammation in the disease.

P.A.S.07.11

The 20s immunoproteasome and the constitutive proteasome bind with the same affinity to PA28αβ and equally degrade FATT10 n. roverato; university of konstanz, konstanz, germany.

The 20S immunoproteasome (IP) is an interferon(IFN)-γ- and tumor necrosis factor (TNF)-inducible variant of the 20S constitutive proteasome (CP) in which all its peptidolytically active subunits β1, β2, and β5 are replaced by their cytokine inducible homologues β1i (LMP2), β2i (MECL-1), and β5i (LMP7). These subunit replacements alter the cleavage specificity of the proteasome and the spectrum of proteasome-generated peptide ligands of MHC class I molecules. In addition to antigen processing, the IP has recently been shown to serve unique functions in the generation of pro-inflammatory T helper cell subtypes and cytokines as well as in the pathogenesis of autoimmune diseases, but the role of IP in immune cell activation remains elusive. In this study we investigated whether the IP differs from the CP in the interaction with two IFN-γ/TNF-inducible substrates: the 11S proteasome regulator PA28αβ and the ubiquitin-like modifier FAT10 (ubiquitin D).

P.A.S.07.12

Progestrone suppresses the inflammatory state of innate cells in peripheral blood and cervical mucous

G. R. Sooranna, N. M. Shah, A. Cocker; N. Singh; M. R. Johnson; Imperial College London, London, United Kingdom.

Introduction: Progestrone (P4) has been shown to be an effective immune-modulator and, in reproductive tissue, P4 suppresses inflammation and maintains uterine quiescence. In clinical practice, P4 supplementation is an effective treatment for the prevention of preterm birth (PTB). Our hypothesis was that P4 skews innate cell phenotype systemically in vivo.

Results: P4 treated women (n=6) showed a significant decrease in peripheral blood classical monocytes (CD14+CD16-) but stable granulocyte proportions. In addition, post P4 treatment CbMC showed a decrease in total neutrophils (p=0.0456), and an increase in apoptotic CD14+ neutrophils (p=0.0313). However, the expression of HLA-DR was unchanged. These data indicate that the collaboration of CD169+ DCs and subsequent T cell activation is dependent on the sialic acid-binding capacity of CD169. Finally, CD8+ T cell responses to vaccinia virus infection are dependent on functional CD169. Together, these data indicate that the collaboration of CD169+ macrophages and CD8+ T cells for the initiation of effective CD8+ T cell responses is facilitated by binding of CD169 to sialic acid containing ligands on CD8α+ DCs.

P.A.S.07.13

Functional CD169 on macrophages mediates interaction with dendritic cells for CD8+ T cell cross-priming

D. van Dinten; H. Veninga; S. Borraz; E. G. Borg; L. Hoogterp; K. Olesek; M. R. Beijer; H. Kalisz; J. Garcia-Vallejo; L. K. Franken; L. B. Cham; K. S. Langi; Y. van Kooyk; D. Aslam; E. G. Borg; L. M. den Hoorn; K. Olesek.

1VUmc Amsterdam, Amsterdam, Netherlands, 2Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain, 3LUMC, Leiden, Netherlands, 4University Duisburg-Essen, Essen, Germany, 5University of Dundee, Dundee, United Kingdom.

Splenic CD169+ macrophages are located in the marginal zone to efficiently capture blood-borne pathogens. Here, we investigate the requirements for the induction of CD8+ T cell responses by antigens (Ag) bound by CD169+ macrophages. Upon Ag targeting to CD169+ macrophages, we show that Batf3-dependent CD8α+ dendritic cells (DCs) are crucial for DNGR-1-mediated cross-priming of CD8+ T cell responses. In addition, we demonstrate that CD169+, a sialic acid binding lectin involved in cell-cell contact, preferentially binds to CD8α+ DCs and that Ag transfer to CD8α+ DCs and subsequent T cell activation is dependent on the sialic acid-binding capacity of CD169. Finally, CD8+ T cell responses to vaccinia virus infection are dependent on functional CD169. Together, these data indicate that the collaboration of CD169+ macrophages and CD8α+ DCs for the initiation of effective CD8+ T cell responses is facilitated by binding of CD169 to sialic acid containing ligands on CD8α+ DCs.

P.A.S.07.14

Modulation of Th17 response in cultures of human peripheral blood mononuclear cells

P. Vidovic1,2; S. Tomic1; 1University of Sarajevo, Medical Faculty in Foča, Foča, Bosnia and Herzegovina, 2University of Defense in Belgrade, Medical Faculty of the Military Military Academy, Belgrade, Serbia, 3University of Belgrade, Institute for Application of Nuclear Energy, Belgrade, Serbia.

Numerous studies performed on purified CD4+ T cells have shown that the cytokine cocktail (IL-1β/IL-6/IL-23) is required for Th17 cell polarization, whereas the role of TGF-β remains controversial. We examined how Th17 response is modulated in the whole peripheral blood mononuclear cell (PBMC) cultures, which better reflect conditions in vivo. PBMC were isolated from healthy volunteers. The cells were cultured under different conditions in the presence of IL-1β/IL-6/IL-23, with or without TGF-β. For stimulation, phytohemagglutinin (PHA) or CD3/CD28 antibodies were used. Some cultures were treated with either IL-10, TGF-β or IFN-γ neutralizing antibodies.
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P.AS.07.15
Complement factors B, F, H and schizophrenia in Armenians
R. Zakharyan1, H. Ghazaryan2, A. Arakelyan1,3
1Russian-Armenian University, Yerevan, Armenia, 2Institute of Molecular Biology NAS RA, Yerevan, Armenia.

Immune system alterations contributed to schizophrenia. We aimed to assess the blood levels of complement factors F (FB) and FH in schizophrenia. According to the results, the FB serum levels were 1.3 times decreased in patients compared to controls (mean±SD,ug/mL: 2.20±0.61 vs 2.76±0.85; p=0.01). No significant difference in the mean FB between untreated patients (48±mg/L) and this finding correlated with the number of cells. The IL-1β and IL-6-23 cocktail significantly augmented the production of IL-17 in unstimulated IL-1β and CD3/CD28-stimulated PBMC cultures, but not in PHA-stimulated cultures. The addition of TGF-β to the cytokine cocktail inhibited the production of IL-17 in all three culture systems. The opposite effect was seen when anti-TGF-β or anti-IFN-γ antibodies were added. It is interesting that anti-IL-10 antibody augmented the percentage of IL-17+ cells, but the production of IL-17 was reduced in the supernatants. In conclusion, our results showed that IL-1β/IL-6-23 stimulated differentiation and activation of Th17 cells in PBMC cultures, but the effect depended on the type of T-cell activation stimulus. The Th17 response was better when TGF-β and Th1-signaling pathways were inhibited. However, the role of IL-10 signaling pathway remains unclear.

P.AS.07.16
Development of novel tools to investigate trogocytosis in T lymphocytes
S. Zenke1, J. Braun, S. Ammann1, N. Beyerstorf1, P. Aicheler, G. Griffiths, J. Rohr1
1Center for Chronic Immune-Suppression, Freiburg, Germany, 2Cambridge Institute for Medical Research, Cambridge, United Kingdom, 3Institute for Virology and Immunobiology, Würzburg University, Würzburg Medical Faculty, Freiburg, Germany.

T cells have long been known to extract surface molecules from antigen-presenting cells – a process termed “trogocytosis”. However, the molecular requirements and biological function of this process is incompletely understood. In order to investigate the mechanisms and function of trogocytosis in T cells we generated cell lines stably expressing an antigen peptide covalently linked to a blue fluorescent pMHC-complex in addition to red fluorescent B7-molecules. Using these artificial antigen presenting cells (APCs) we find that naïve CD8+ T cells rapidly and efficiently acquire peptide-MHC complexes and B7 molecules from artificial APCs. This process is dependent on specific interactions between the T cell receptor/peptide-MHC and CD28/B7. Transfer of B7 does not require specific TCR engagement, but concomitant pMHC-recognition increases the efficacy of B7 transfer. Furthermore, we show that a substantial part of trogocytosed molecules can be re-expressed on the T cell surface – a finding that we confirmed to also occur in murine models in vivo. Currently, we are investigating specific cellular function to reveal the molecular mechanisms underlying trogocytosis in T cells. In summary, trogocytosis constitutes a mechanism how HLA class II cell surface molecules can efficiently acquire and re-express surface molecules from neighbouring cells – thereby equipping them with the armamentarium of bona-fide APCs.

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P.AS.07.18
Metabolic mimicry of regular high-intensity exercise modulates neutrophil function
J. Sanchez, S. Walker, A. Gutierrez del Arroyo, G. L. Ackland
University of Bristol, University of London, London, United Kingdom

Introduction
Regular high-intensity exercise is associated with longevity and improved health. Modulation of innate immunity may contribute to the positive impact of exercise, although the mechanisms remain unclear. High-intensity exercise is characterised by accumulation of lactate as a product of anaerobic glycolysis. We examined whether mimicking repeated high-intensity exercise through direct, repeatedrogenous administration of lactate may modulate innate immune responses.

Methods
C57BL/6 mice were randomly to receive injected intraperitoneal (200μl) injections of either saline or sodium lactate to mimic high-intensity exercise on 5 consecutive days. 24h after the last pre-treatment, zymosan (1mg) was injected to elicit neutrophil influx into the peritoneum. Peritoneal lavage and bone marrow were harvested either 2.5h or 5h later. Neutrophil CD11b expression and ex vivo phagocytic activity of Staphylococcus aureus bioparticles by peritoneal neutrophils was assessed by flow cytometry.

Results: More CD45+CD11b+Ly6G+ cells (11.6±2.3±7.4×10^6/ml) were present in the peritoneum after sodium-lactate pre-treatment, compared to saline (3.9±6.1±13±10^6/ml; p<0.01; group p<0.05). The expression of CD62L, CD11b and CD69 in peritoneal neutrophils showed CD11b expression (median fluorescence intensity (MFI): 327.7±164.2), compared to saline pre-treatment (MFI: 766.2±167.0; p<0.005). Sodium-lactate pre-treatment was associated with a reduction in peritoneal neutrophil CD11b expression (median fluorescence intensity (MFI): 89.6±110.4 vs 144.1±105.0, p<0.001), whereas a significantly increased 402H level compared to 402H in controls was observed (265.6±28.4 vs 79.6±71.9, p<0.0001). These results suggested the important role of FB and FH in schizophrenia. Further replication studies are needed to confirm these findings.

P.AS.07.19
Changes in the T cell receptor repertoire during pre-treatment with dexamethasone in paediatric HIV
T. C. Attneburough1, K. Schoo Sanggaard2, B. Margetti3, S. Adams1, R. Callard1, A. Glazi Soragoi, N. Kleir1
1UCL Great Ormond Street Institute of Child Health, London, United Kingdom, 2Great Ormond Street Hospital for Children, London, United Kingdom

Antiretroviral therapy (ART) is generally very effective in children infected with HIV, and they can often recover from the depletion caused by the disease. However side effects can sometimes lead to medication nonadherence or medical recommendation for a treatment break. Studies have already shown that CD4+ cell levels are generally restored with ART reintroduction. This study used Next Generation Sequencing (NGS) techniques to examine the impact of ART interruption and reintroduction on the T cell receptor repertoire (TCR) repertoire in great detail.

We used NGS to estimate the TCR repertoire from 4 paediatric patients living with HIV. The samples were accessed from a randomised controlled trial and span before, during, and after a 48 week ART treatment interruption. The samples were purified to collect the naive CD4+ T cells and memory CD8+ T cells from each sample.

We found similarities in the TCR repertoire profiles before the ART interruption and post reintroduction. We were able to track specific CDR3 sequences over time. A large number of CDR3 sequences were also shared between patients, and showed several different patterns over the time.

The similarity of the TCR repertoire profiles at the beginning and end of the study suggests that in general, the treatment interruption in paediatric patients doesn’t appear to have long term negative effects on the TCR repertoire. We also found CDR3 sequences in both naive CD4+ T cells and memory CD8+ T cells that were shared between patients.
T. Dyskova, V. Smotkova-Kraiczova, J. Gallo, E. Kringova;

1. Dept. of Immunology, Palacky University, Olomouc, Czech Republic, 2. Dept. of Orthopaedics, Palacky University/University Hospital, Olomouc, Czech Republic.

Introduction: Individual susceptibility to periprosthetic joint infection (PJI) overall total hip arthroplasty (THA) is associated with genetic variations in cytokine genes. Thus, cytokine patterns may differ in THA patients with mild and severe PJI and in patients carrying a risk allele TNF-238A, associated with severe osteolysis. Methods: Peripheral blood mononuclear cells (PBMCs) obtained from 31 THA patients with severe (n=23) and mild (n=8) osteolysis were stimulated with PMA/Ionomycin, CD3/CD28, LPS, CpG ODNs (K- and D-types). The IL-4, IL-5, IL-10, IFN-γ, TNF-α, VEGF, and RANKL were measured by ELISA. Results: Elevated expression of IL-2, IL-5, VEGF, and RANKL (P<0.05) was observed in supernatants from patients with severe comparing to mild osteolysis, irrespective of TNF-238 genotype. Patients with TNF-238 GG genotype with severe osteolysis showed up-regulated expression of all studied cytokines, except IL-10 and VEGF, comparing to those with mild osteolysis. In severe osteolysis, the carriers of rare TNF-238A allele showed lower expression of IL-2, IFN-γ, and RANKL (P<0.05) when comparing to non-carriers. Conclusion: Alteration in cytokine expression pattern in LPS-stimulated PBMCs from THA patients with mild and severe osteolysis and differing by TNF-238A genotype was observed. Molecular mechanisms by which TNF-238A allele increases a risk of severe osteolysis should be further investigated. Grant support: M2 CZ VR16-31852A, MZ Dr VES1-27726A, IGA UP 2018_016, MH CZ - DRO (FNOL, 00098892).

P.A6.01.05

TLR Dependent Immune Responses of Patient Displaying Common Variable Immunodeficiency Like Symptoms

I. Evcili, G. G. Kaya, M. Yıldırım, N. Bozbeyolu, I. C. Ayanoglu, M. Gürsel, I. Gürsel;

1. Bilkent University, Ankara, Turkey, 2. Middle East Technical University, Ankara, Turkey, 3. Ege University Faculty of Medicine, Izmir, Turkey.

1. Bilkent University, Ankara, Turkey, 2. Middle East Technical University, Ankara, Turkey, 3. Ege University Faculty of Medicine, Izmir, Turkey.

The innate immune system uses germline coded receptors to detect pathogens and mount acute innate response that helps to protect host from infection. Toll-like receptors (TLRs) and other pathogen dependent pattern recognition receptors play important role in this response. Common Variable Immunodeficiency (CVID) is the most common primary immunodeficiency seen in adults. Vitamin D and VDR have been reported to have an effect on TLR2. In this study, PBMCs from CVID-susceptible VDR+ patient was treated with TLR and inflammation-stimulated ligands (Pam3CysK4, P2-C, LPS, R484, Resiquimod, Cnt (Bacteria, Neurovir and Antigen) for 24h. IFN-γ, IL-4, IL-5, Pan-IFNα and IP-10 levels were determined from the supernatants by ELISA. Total RNA isolated from PBMCs was studied with Nanostring™ inflammation panel. Th1/Th2/Th17 phenotyping was performed by flow cytometry and IP-10 and IL-1B levels in plasmas were determined by CBA. IFN-γ and IL-10 levels were significantly higher than healthy levels. The inflammation panel analyses via Nanostring™ revealed that TLR2, TLR4, TLR7/8, NF-κB, Stat1, IL-15 levels were magnified compared to healthy PBMCs. TLR pathway analyses implicated that R7K gene level was increased, whereas mRNA levels of other cytokines were lower compared to healthy PBMCs. The chemokine network analyses suggested an increased expression of IP-10, CXCL9, CXCL10, and CXCL11. Strikingly, chemokine receptors were decreased. Our investigation implied that TLR pathways responses could be worsen during ongoing active infection.

P.A6.01.02

Late onset of maternal CD8 T-cells GVHD in a toddler with JAK3 severe combined immunodeficiency


1. Bilkent University, Ankara, Turkey, 2. Middle East Technical University, Ankara, Turkey, 3. Ege University, Izmir, Turkey.

Recurrent osteolysis seen in adults. Vitamin D and VDR have been reported to have an effect on TLR2. In this study, PBMCs from CVID-susceptible VDR+ patient was treated with TLR and inflammation-stimulated ligands (Pam3CysK4, P2-C, LPS, R484, Resiquimod, Cnt (Bacteria, Neurovir and Antigen) for 24h. IFN-γ, IL-4, IL-5, Pan-IFNα and IP-10 levels were determined from the supernatants by ELISA. Total RNA isolated from PBMCs was studied with Nanostring™ inflammation panel. Th1/Th2/Th17 phenotyping was performed by flow cytometry and IP-10 and IL-1B levels in plasmas were determined by CBA. IFN-γ and IL-10 levels were significantly higher than healthy levels. The inflammation panel analyses via Nanostring™ revealed that TLR2, TLR4, TLR7/8, NF-κB, Stat1, IL-15 levels were magnified compared to healthy PBMCs. TLR pathway analyses implicated that R7K gene level was increased, whereas mRNA levels of other cytokines were lower compared to healthy PBMCs. The chemokine network analyses suggested an increased expression of IP-10, CXCL9, CXCL10, and CXCL11. Strikingly, chemokine receptors were decreased. Our investigation implied that TLR pathways responses could be worsen during ongoing active infection.

P.A6.01.03

A novel splicing mutation in a patient with Griscelli syndrome type 2


Introduction: Autosomal severe combined immunodeficiency (SCID) due to Janus kinase (JAK) 3 deficiency is characterized by a T+B+NK- phenotype. Objective: To describe a delayed presentation of maternal CD8+ engraftment in a 15-month-old boy associated with fever, failure to thrive, hepatomegaly and atopic dermatitis. Prior to his referral he had been treated with CMV vaccines (PVR max 1500 IU/ml). Not receiving antiviral treatment and MMp vaccine given aged 12 months was not associated with complications. Family history revealed parental consanguinity and a sister died at 7 months due to infectious complications. Material and methods: Immunophenotype using flow cytometry in peripheral blood, quantitative of immunoglobulins and next generation sequencing of the genes related to SCID were performed. Results: Lymphocyte subsets showed absent levels of CD4+ T-cells, decreased NK-cells, normal CD8+ T-cells and elevated B-lymphocytes. IgG and IgM levels were normal for age (IgA absent). T-cell receptor excision circles (TRECs) and kappa deletion recombinase excision circle (KRECS) of the dried blood sample were 0/punch and 31/punch (normal >6 and >4/punch, respectively). Maternal CD8+ engraftment was confirmed using HLA-DR typing. The variant c.2892G>T (p.Lys964Asn), not previously described, was found to be homozygous in the JAK3 gene and confirmed by Sanger sequencing. Conclusions: We describe a child with a combined immunodeficiency and late onset of maternal CD8+ GVHD due to a new homozygous mutation in JAK3. Interesting the toddler did not suffer from disseminated CMV infection nor live vaccine associated complications to be expected in this immunocompromised host, probably due to the "protective" effect of maternal CD8 cells.
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206

P.A.6.01.06
Systemic homeostasis reveals autophagy-dependent control of adaptive immunity in the gut


Complex autoimmune diseases have a strong genetic component. While genome-wide association studies have been very successful at identifying genetic determinants of large effect size, these studies run into limitations both in the identification of low effect size variants, and in the functional interpretation of identified non-coding variants. This creates a gap of unexplained genetic contribution that has termed missing heritability. Mouse genetic studies aid in the search for new genetic determinants by offering stable experimental conditions and the possibility for functional characterization. In this study we identified a non-coding simple repeat that reduced disease severity in mouse models of arthritis, multiple sclerosis, and delayed-type hypersensitivity in a sex-specific manner. We found that the simple repeat interfered with DNA-binding of the transcriptional regulator CTCF by creating a base pair change AC to GG within the CTCF consensus motif. As a consequence, it affected the expression profile of nearby genes, including the TCR co-stimulator Cd27, changing the reactivity of peripheral T cells. We found that these changes in immune function and disease progression depended on sex hormones. Indeed, castration of female mice both reduced binding of CTCF to the candidate site, and was sufficient to reverse the protective effect in the EAE model of multiple sclerosis. In conclusion, we identified a non-coding simple repeat that dictates the expression profile of surrounding genes in a sex-dependent manner, culminating in a sexually dimorphic autoimmune phenotype.

P.A.6.01.07
Genetic study in a Spanish cohort of narcolepsy type 1 patients and susceptibility to autoimmune diseases


Introduction. Narcolepsy Type 1 (NT1) affects between 0.025-0.40% population in Europe. This chronic sleep disorder is characterised by excessive daytime sleepiness, cataplexy and disturbed nocturnal sleep. It is caused by a selective loss of brainstem cortically projecting neurons that indicates an autoimmune pathogenesis. There is also a strong association with HLA genes, as the 82% of patients display the DRB1*06:02 allele. The purpose of this study is to analyse the role of the HLA genes in NT1 patients and the susceptibility to autoimmune diseases. Material and methods. A total of 62 DNA samples from NT1 patients were analysed. Each DNA was purified through MagnaPure automatic technology (Roche®, Switzerland). The DNA quantification was done through Nanodrop spectrophotometer (Thermo Fisher Scientific®, USA) and HLA typing was done via Lenogen long-range PCR. Results. S4 NT1 patients out of 62 (81.6% in NT1 vs. 22% in controls, P<0.01) had the DRB1*06:02 allele. Moreover, 22 NT1 patients out of 62 (35.48%) had one or more autoimmunity diseases (AD) associated. Within this group, 18 displayed the DRB1*06:02 allele (81.8% in NT1 vs AD vs. 39.2% in controls, P<0.01), whereas 4 patients were DQ2 positive because the DR3 allele was significantly more frequent than in controls (75% in NT1 with 9.5% vs. 21% in controls, P<0.001). Conclusion. The DRB1*06:02 allele plays an important role in NT1 patients. Furthermore, there is a high frequency of ADs in NT1, which suggests that narcolepsy has an autoimmune pathogenesis.

P.A.6.01.08
Celiac Disease: a dose-dependent effect of HLA-DQB1*02 gene to genetic susceptibility in paediatric patients

M. Fernandez-Arquero, L. N. Campo Blazquez, F. Genel Fernandez, A. Bados, A. Garcia-Rio, M. T. de las Santas, K. Guerra, S. Sanchez-Ramon, C. Alonso; San Carlos University Hospital, Madrid, Spain.

Introduction: Celiac disease (CD) is a T cell-mediated, tissue-specific autoimmune disease which is found in genetically susceptible individuals who carry the HLA-DQB1*02 or DQA1*0501 genes. This pathology causes damage to the small intestinal mucosa when gluten or related prolamines, are ingested. In these patients, the small bowel biopsies have been widely accepted as a gold standard for diagnosis. However, it is clearly known the effect that HLA-DQB1*02 gene does in predisposition to CD. The aim of this study is to analyse the role of the HLA-DQB1*02 genes in paediatric patients. Material and methods: 200 DNA samples were analysed from CD diagnosed patients (100) and healthy donors as controls (100). Each DNA was obtained with MagnaPure automatic technology (Roche®, Switzerland). The DNA quantification was done through Nanodrop spectrophotometer (Thermo Fisher Scientific®, USA) and HLA typing was performed using Lenogen long-range PCR (Genetica LongWood®, Spain). Results: We observed 92% of the patients displayed the DQA1*0501 and DQB1*02 alleles, in contrast to controls: 25% (p<0.0001). The DR7, DQ2 and/or D4, DQ8 haplotypes, were present in those CD patients that didn’t carry the Di QB1 heterodimer. Moreover, a 58% of patients displayed the DRB1*07 allele carrying DQ2 haplotype, in contrast to controls: 3.3% (p<0.0001). Conclusions: We remark the role of DQB1*02 as a strong marker of genetic predisposition in paediatric patients, which could be used as possible prognostic factor of CD. The presence of DRB1*07, agree with an additive effect of DQ2 haplotype to that conferred by the DQ2.5 when these are present.

P.A.6.01.09
Heterozygous mutation in BCL10 in a patient with T lymphopenia and ulcerative colitis

S. Garcia Gomez1, L. Trotta1, R. Martinez-Barricarte1, Y. Haro1, T. Martelius1, B. Boisson1, S. Sanchez-Ramón1, E. Lopez-Collazo1, M. Martin Arranz1, E. Martin Arranz1, L. Garcia-Ramirez1, J. Saarela1, J. Casasnovas1, M. Sepulveda1, R. Perez de Diego1,2,3,1Laboratory of Immunogenetics of Diseases, IDiPaz, Institute for Health Research, Madrid, Spain; 2Innate Immunity Group, IDiPaz Institute for Health Research, Madrid, Spain; 3Institute for Molecular Medicine Finland, Helsinki, Finland; 4St.Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, United States; 5The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York, United States; 6Adult Immunodeficiency Unit, Mucosal Inflammation Center Helsinki University and Helsinki University Hospital, Helsinki, Finland; 7Clinical Immunology Department, San Carlos Clinical Hospital, Madrid, Spain; 8Innate Immunity Group, IDiPaz Institute for Health Research, La Paz Hospital, Madrid, Spain; 9Gastroenterology Department, La Paz Hospital, Madrid, Spain; 10Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland; 11Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland; 12Howard Hughes Medical Institute, New York, United States; 13Laboratory Of Human Genetics Of Infectious Diseases Necker Branch, Imagine Institute, Necker Hospital for Sick Children, Paris, France; 14Adult Immunodeficiency Unit, Mucosal Inflammation Center Helsinki University and Helsinki University Hospital, Helsinki, Finland; 15Rare Disease Cebterm Children’s Hospital Helsinki University and Helsinki University Hospital, Helsinki, Finland.

Capase recruitment domain-containing (CARD) family adaptors form heterotrimers with B-cell lymphoma 10 (BCL10) and mucosa-associated lymphoid tissue lymphoma-translocation gene 1 (MALT1). CARD-BCL10-MALT1 (CBM) complex activates nuclear factor (NF-kB) in both the innate and adaptive arms of immunity. Inherited defect of human BCL10 complete deficiency was recently reported in a child with a broad combined immunodeficiency. This patient suffered a chronic non-specific colitis with moderated lymphocytic infiltration in lamina propria. We report here a new mutaton of BCL10. The patient carrying the mutation is an adult with chronic primary T lymphopoenia, low B cell levels and ulcerative colitis. A heterozygous mutation in BCL10 was found (L8P). In spite of normal BCL10 expression, this patient has an impaired NF-kB-mediated function in fibroblast similar to the one observed in the first patient reported. Our results show that BCL10 L8P heterozygous mutation can be responsible of this new phenotype.

P.A.6.01.10
A rare cause of immunodeficiency: glycogen storage disease type 1b

B. Erdur, I. Parlak, M. Köse, E. Şahin, E. Özbek, F. Genel; Department of Pediatrics Dr.Behcet Uz Children’s Hospital, Izmir, Turkey.

Glycogen storage diseases (GSD) are inherited metabolic diseases characterized by accumulation of glycogen in tissues. GSD type 1b effects lipid, carbohydrate and purin metabolism and neutropenia may occur. The patients are susceptible to recurrent bacterial infections and have recurrent oral ulcers due to immune dysfunction caused by a combination of neutropenia and impaired phagocytic functions. Here we present an infant with GSD type 1b admitted for failure to thrive, abdominal distention, and developed hypoglycemia during the follow up. We remark the expression profile of nearby genes, including the TCR co-stimulator Cd27, changing the reactivity of peripheral T cells. We found that these changes in immune function and disease progression depended on sex hormones. Indeed, castration of female mice both reduced binding of CTCF to the candidate site, and was sufficient to reverse the protective effect in the EAE model of multiple sclerosis. In conclusion, we identified a non-coding simple repeat that dictates the expression profile of surrounding genes in a sex-dependent manner, culminating in a sexually dimorphic autoimmune phenotype.
Posters

P.A6.01.11
Innate immune responses in MHC class I deficiency resulting from β2-microglobulin gene mutation

B. Kaygaard1, N. Sürçiç2, A. Edert, H. Uçpınar1, M. Özyapı1, I. C. Ayayorglu1, B. Geckin1, A. M. Acar1, Ö. Ardeniz1, M. Günsel1

1Department of Biologic Sciences, Middle East Technical University, Ankara, Turkey, 2Medicine Division of Allergy and Clinical Immunology, Ege University Medical Faculty, Izmir, Turkey.

Introduction: A novel mutation in β2-microglobulin gene has been recently identified in two Turkish siblings, resulting in low numbers of CD8 T cells and absence of MHC class I expression. Although such a phenotype would be expected to result in susceptibility to mainly viral infections, the 26-year-old male patient (one of the siblings) was fairly asymptomatic compared to his sister, who suffered from multiple forms of immune dysregulation. Herein, we aimed to investigate the underlying cause of innate immune system hyperactivation by means of functional assays.

Methods: PBMCs from patient and healthy donors were stimulated with various pattern recognition receptor (PRR) ligands and immune responses were analyzed using cytokine ELISA. STAT phosphorylation and cytotoxic ROS production were assessed by flow cytometry. Gene expression was analyzed by Nanostring inflammation panel.

Results: Stimulation of cells with various PRR ligands resulted in diminished pro-inflammatory cytokine production in the patient as opposed to an increase in type I IFN secretion when compared to healthy controls. Patient PBMC spontaneously secreted IFN-10 in the absence of stimulation. Nanostring gene expression analysis was consistent with a pro-inflammatory pattern level analysis, indicative of enhanced type I IFN signature. In addition, the patient’s neutrophils were observed to produce spontaneous ROS and this patient was found to have high percentage of low density neutrophils (LDG) that was absent in healthy individuals.

Conclusion: Presence of hyperactive neutrophils and LDGs may explain resistance to bacteria while an increased type I IFN signature may explain the resistance to viral infection in this patient.

P.A6.01.12
The NGS approach in evaluation of known and novel mutations associated with X-linked agammaglobulinemia (XLA)

I. Kofadi1, I. Manto1, E. Latysheva2, T. Latysheva2, A. Nikiforova2, G. Gudima1, M. Khaivo11

1FSBI “NRC Institute of Immunology” FMBA of Russia, Moscow, Russian Federation, 2DNA-Technology, ISC, Moscow, Russian Federation.

The XLA specified as primary humoral immunodeficiency with apparent genetic component. The disease predominantly manifested in patients bearing mutations in BTK gene and characterized by the failure to produce mature B-lymphocytes. The differential diagnosis from other PIDs, characterized by similar symptoms, but requiring other treatment, is currently challenging without genetic confirmation. Currently the most effective and relevant approach for target genetic screening is NGS. We developed the test system covering 19 exons of BTK gene suitable for Illumina MiSeq platform. The study enrolled 7 patients with possible XLA (ESID criteria) between 18 and 36. 4 patients showed single pathogenic mutations. One patient had 3 different mutations and another one had 4 different mutations. In addition, one patient with apparent symptoms of XLA had no mutations in BTK gene. The results were compared with most relevant and complete databases: BTKbase and LOVD, as well as checked for citatation in PubMed. Analysis showed that 5 mutations were not indexed in databases. Two of them NM_000601.2:c.241-1G>A and NM_000601.2:c.1178-1G>A were previously described by Stewart D.M. (2001) and Toth B. (2009) respectively. Three mutations could be considered to be new, and presumably pathogenic: two deletions -NP_000052.1:p.Met57del/ NM_000601.2:c.1708_1710delATG; p.Cys145AlafsX31/ NM_000601.2:c.433delT and one SNP - NM_000601.2:c.1909+1G>A. The obtained results confirm the utility of NGS approach in evaluation of BTK mutation profile. The genetic data are presented in further follow-up and characterization of 1-cell immunity of XLA patients. Non-confirmed diagnosis will be reconsidered and subject to further investigation.

New mutations will be submitted to BTK mutation databases.

P.A6.01.13
Characterization of the monocyte/macrophage compartment in a patient with a novel CSF1R mutation causing hereditary diffuse leukocytephagocytosis with spheroids

D. Quandt1, T. Krakow, T. Pifflmann1, A. Kindermann1, J. Kohliase, D. Stoeseandt, K. Hoffmann1, P. Villaviciencio-Lorin2

1Department of Anatomy and Cell Biology, University of, Halle, Germany, 2Department of Neurology, University of, Halle, Germany, 3Institute of Pharmacological Chemistry, University of, Halle, Germany, 4SYNLAB MVZ Humangenetik Freiburg, Freiburg, Germany, 5Department of Radiology, University of, Halle, Germany, 6Institute of Human Genetics, University of, Halle, Germany.

The transcription factor nuclear factor kappaB (NFκB1) is a tyrosine kinase transmembrane protein that mediates proliferation, differentiation and survival of monocytes/macrophages and microglia by activation through the cytokine CSF1 or IL-34. Interestingly, CSF1R gene mutations cause hereditary diffuse leukocytephagocytosis with spheroids (HDLS), an autosomal-dominantly inherited myeloprophagocytosis leading to rapid neurocognitive decline with high lethality. By detailed clinical assessment and targeted gene sequencing we identified a novel CSF1R-indel mutation in a 44-year old female patient with a complex neuropsychiatric clinical presentation, initial signs of cerebral gliosis, and positive family history. By FACS analysis of peripheral blood monocytes we detected marginal elevated cell surface expression levels of the CSF1 (ED1/115) receptor. Interestingly, we found an increased number of total blood monocytes. Subdividing monocyte populations by flow cytometry revealed a decreased frequency of non-classical monocytes in the blood of the patient. Of particular note, we discovered an increased Tyr272 autophosphorylation by intracellular flow cytometry in peripheral monocytes, indicating a gain-of-function effect of the mutation. Ongoing analyses will reveal whether there is an altered potential of macrophage differentiation and polarization. Furthermore pharmacological inhibition of the CSF1R by the use of GWS2580, shown to arrest microglial proliferation, will be applied in vitro to study potential treatment options for this novel CSF1R-indel mutation.

P.A6.01.14
Investigation of heterozygous NFKB1 variants in a common variable immunodeficiency cohort

C. Schröder1, T. Witter, T. Jacob1, T. Đakí, B. Grimnbacher2, R. E. Schmidt, F. Atschekzei2

1Hannover Medical School, Hannover, Germany, 2Center for Chronic Immunodeficiency, Freiburg, Germany.

The transcription factor nuclear factor kappa B (NF-kB1) is sequestered within the cytoplasm of every cell. During activation of the canonical NF-kB pathway, NF-kB1 translocates to the nucleus and binds to the promoters of its target genes. NF-kB1 has been linked with a diversity of diseases, including asthma, AIDS, diabetes and cancer. More recently, it has been shown that NFKB1 mutations could lead to haploinsufficiency of the activating unit p50 and therefore could cause the VIDD1 phenotype. CVID as a syndrome comprises a heterogeneous group of molecular diseases, characterized by a significant hypogammaglobulinemia of unknown cause. Genomic DNA for targeted NGS was isolated from whole blood. Detected mutations were validated by Sanger sequencing. PBMCs were isolated by density gradient centrifugation and stimulated with PMA plus ionomycin and analyzed using immunoblotting with antibodies against NF-κB1 p105 and p50. In our study we identified five novel heterozygous mutations in NFKB1 in seven patients by targeted NGS. Among those, one frameshift deletion, three single base-pair insertions, one missense and one splice site mutation were observed. NFKB1 mutations occur in our CVID cohort with a prevalence of 1.30. In all affected members of three families, their mutations lead to a reduced level of the active NF-kB1 subunit p50. Nevertheless, the mutations segregate with incomplete penetrance in families. Mutations in NFKB1 lead to reduced level of p50. Due to the incomplete segregation of penetrance, other causes, like epigenetic changes or intestinal microbicide alteration may promote the onset of disease. Supported by DZIF TLU 07.801

P.A6.01.15
Molecular and bioinformatics characterization of a novel mutation in STXBP2 gene: a case report on Familial Hemophagocytic Lymphohistiocytosis

L. Viljas-Gimenez1, E. Donadeu4, E. Alvarez de la Campa3, I. Calobran1, A. Catalá1, X. de la Cruz1, L. Alsin1, J. Sayos2, M. Martinez-Gollci1, A. M. Acar3, J. Sayos2, J. Sayos2

1Vall d’Hebron University Hospital, Barcelona, Spain, 2Institut de Recerca Vall d’Hebron (VHIR), Barcelona, Spain, 3Hematology Department, Hospital Sant Joan de Déu., Barcelona, Spain, 4Hematology Department, Hospital Sant Joan de Déu., Barcelona, Spain.

Familial Hemophagocytic Lymphohistiocytosis (FHL) is a rare autosomal recessive disorder characterized by uncontrolled immune activation but ineffective response. Disease-causing mutations have been reported in several genes: PRF2-FHL2, UNC13D-FHL3, STX11-FHL4 and STXB2-FHL5. All ILH forms are diagnosed based on clinical symptoms and laboratory findings following accepted guidelines. We present a 2-year-old boy-baby from non-consanguineous parents that developed two episodes of EBV-HLH in 4 months. His older brother died at the age of 3 years of HLH also triggered by EBV infection. Functional test revealed impaired cytotoxicity, reduced CD107a Mean-Fluorescence-Intensity (MFI) but normal percentage of NK cells expressing CD107a. Genetic analysis identified a compound heterozygous mutation in STXBP2 gene, one a previously reported in exon 15 (c.1247-1G>C) and the second a novel mutation in exon 9 (c.287T>A) with PMDD and PMLD prediction. The L243 residue is evolutionarily highly preserved and PyMOL prediction indicates that sits at the centre of a rich network of interactions important for stabilizing domains 2 and 3 of the STXB2 protein. Transfection of constructs with the mutated and WT sequences into COS7 cells resulted in good level of WT but no expression of STXB2-L243R, confirming PyMOL prediction. Overall the known effect of the allele p.V417LfsX126 plus the in-silico analysis and transcription experiments of the L243R allele indicate that all the remaining degranulation function of STXB2 is attributable to the p.V417Lfs allele. This would explain the results of functional assays, i.e., impaired cytotoxicity with reduced CD107a surface expression as assessed by MFI but normal as percentage of CD107a+ NK cells after stimulation.
POSTER PRESENTATIONS

P.A6.01.16
Clinical and Immunologic Data in Two Groups of Familial and Sporadic Patients with Common Variable Immunodeficiency

R. Yazdani, A. Valsalam, G. Azizi, H. Abohlouzami, A. Aghamohammadi 1
Research Center for Immunodeficiencies (RCID), Tehran, Iran, Islamic Republic of.

Introduction: Common variable immunodeficiency (CVID) is the most frequent symptomatic primary immunodeficiency disease and its prevalence varies significantly among different population. Minority of CVID patients present a familial aggregation suggesting a higher probability of heritable genetic defects. Methods: A total of 235 registered CVID patients were evaluated in this cohort study. Familial and sporadic patients were stratified and demographic information, clinical records, laboratory and molecular data were compared among these two groups of patients. Results: Multiple cases were identified in 12 families (30 patients) and sporadic presentation in 120 cases. The rate of parental consangiunity (83.3%) and clinical presentation of lymphoid malignancy (20.7%) were predominant in familial CVID patients, whereas significantly increased recurrent upper respiratory infections were recorded in sporadic patients (3.0% patients per year). Proband of familial group were presented with a higher severity score resulting in a profound mortality rate (41.7% after 30-years follow-up) comparing to the non-proband CVID patients in the same families with a lowered diagnostic delay. Conclusion: Familial CVID patients had a more severe phenotype and clinical presentation and immunologic profile and a high consangiunity in this group of patients suggests a Mendelian trait with an autosomal recessive inheritance pattern. Diagnosis of an index patient within a multiple case families significantly improves the diagnostic process and outcomes of the yet asymptomatic patients.

P.A6.01.17
First case with cernunnos deficiency from the national Iranian registry

R. Yazdani, H. Abohlouzami1, J. Tafarangi, G. Azizi, A. Hamidieh, J. Chou, R. S. Geha, A. Aghamohammadi; 1Research Center for Immunodeficiencies (RCID), Tehran, Iran, Islamic Republic of, “Division of Immunology Boston Children’s Hospital, and Department of Pediatrics, Harvard Medical School, Boston, USA, Boston, United States.

Introduction: Severe combined immunodeficiency (SCID) is a heterogeneous group of genetic disorders. Cernunnos is a DNA repair factor that is involved in Non-homologous end-joining (NHEJ) process. Impairment in Cernunnos leads to a genetic disease characterized by neural disorders, immunodeficiency and increased radiosensitivity. Methods: We obtained clinical manifestations and immunological findings by reviewing hospital records. Mutation analysis was done by whole exome sequencing and the mutation was confirmed by sanger sequencing. Results: We herein describe a SCID patient with T+B phenotype who had a mutation in Cernunnos gene and manifested recurrent infections, microcephaly and growth retardation with hypogammaglobulinemia. Furthermore, our patient was associated with BCG adenitis and autopsmy that is less observed in patients with Cernunnos deficiency. Conclusion: In contrast to previous reported Cernunnos-deficient patients, our patient had normal B-cell along with normal IgA and IgM, suggesting a leaky form of Cernunnos deficiency due to residual count of B cells in our patient. Cernunnos deficiency should be considered in children with recurrent bacterial infections, microcephaly and growth retardation, in spite of having normal B cell as well as normal IgM and IgA level.

P.A6.02 Lessons learned from the genetic defects - Part 2

P.A6.02.01
Association of interferon regulatory factor (IRF 5) gene (rs2280714) SNP with systemic lupus erythematosus patients

H. Hamidi, A. Waqar, S. Ullah, A. Jamal, S. Varzaif, S. Janh, N. Afzal, B. Adibi; 1ICBS, Faculty of Health and Allied Sciences, Lahore, Pakistan, 2King Edward Medical University, Lahore, Pakistan, 3University of Health Sciences, Lahore, Pakistan, 4Faculty of Health and Allied Sciences, Lahore, Pakistan.

Background: Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disorder. Center for disease control (CDC)-Atlanta estimated 1.8 to 7.6 per 100,000 people affected with SLE per year in the USA. SLE is a heterogeneous disease. Various genome wide association studies have shown association of interferon regulatory factor 5 (IRF5) gene with SLE. Therefore, this study was aimed and designed to determine single nucleotide polymorphism (SNP) in IRF5 gene restriction site (rs2280714) in local SLE patients and healthy controls. Objective: To determine the frequency IRF5 (rs2280714) gene polymorphism in SLE patients and healthy controls Materials and methods: It was a case control study. Eighty samples were recruited for each of the two study groups. DNA extraction was carried out using standard phenol-chloroform technique. Further, samples were processed by PCR-RFLP (Restriction fragment length polymorphism) conventional method. Polymorphism analysis and allele frequencies were compared between groups using chi-square test. Results: It revealed that SNP in IRF5 gene (rs2280714) is not associated with SLE in Pakistani population. CC genotype is more frequent among various major clinical manifestations of SLE. Conclusion- This study might incorporate with even better clarification of underlying etiological and prognostic factors regarding SLE.

P.A6.02.02
Identification of Tyk2 loss-of-function mutations in a cohort of B cell acute lymphoblastic leukemia patients and characterization of B cell dysregulated function in TYK2- deficient mice

I. Bodega-Mayor1, E. A. Turniari2, I. Cortegano2, M. L. Gaspar1, B. de Andrade3, E. Fernandez-Ruiz; 1Instituto de Investigación Sanitaria Princesa (IIS-IP), Madrid, Spain, 2Centro Nacional de Microbiología, Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

B-cell acute lymphoblastic leukemia (B-ALL) is the most prevalent childhood hematological malignancy. In these patients, mutations in genes associated with JAK/STAT signaling are frequent. Tyrosin kinase 2 (TYK2) is a member of the Janus kinase family (JAKs), involved in several cytokine signaling pathways, and therefore it is important in hematopoiesis and immune system. TYK2-null mouse show deficient tumour surveillance and resistance to LPS-induced septic shock, however the implication of B cells in these phenotypes is still unknown. Using next-generation sequencing, we have sequenced DNA from 65 B-ALL patients at diagnosis and identified two mutations not previously reported and eight polymorphisms in TYK2. We have tested in vitro these new mutations and one polymorphism, and they showed an impaired signaling in response to IFN-alpha, resembling the kinase-dead form of the protein. Additionally, we have studied the B cell compartment of TYK2-null mice after in vivo immunization. We have observed an altered humoral B cell response to T-dependent antigen (NP-OVA), with a significant reduction of memory switched B cells and specific IgM and IgG2a sera. These mice respond also deficiently after the T-independent stimulation with LPS, by decreasing the total number of marginal zone B cells and increasing follicular B cells.

P.A6.02.03
Functional characterization of disease associated variants of human complement factor H-related protein 5

M. Cuerhmali1, B. Uszonyi1, D. Csuka1, K. Uray1, A. Ilais2, Z. Prohaska1, M. Ilais2; 1Department of Immunology, Eötvös Loránd University, Budapest, Hungary, 23rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary, UMTA-ELTE Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Loránd University, Budapest, Hungary.

Complement is a major humoral arm of innate immunity that plays important roles in the protection against infections, regulation of inflammation and disposal of immune complexes and cellular waste. Dysregulation of the complement alternative pathway is involved in the pathogenesis of several diseases, including the kidney disease C3 glomerulopathy. Factor H is a main inhibitor of the alternative pathway; however, the role of the factor H-related FH-related proteins is less characterized. FHRS, which consists of 9 complement control proteins (CCP) domains, was described to bind C-reactive protein (CRP) and C3b. Our aim was to map the ligand binding sites in FHRS and characterize FHRS variants described in patients. Wild type and mutant FHRS were expressed in insect cells. Binding studies were performed by ELISA and surface plasmon resonance (SPR). Fifteen amino acid-long peptides of FHRS CCPs 3-9 and mutant peptides were synthesized on Mimotopes NCP gears. SPR experiments showed that FHRS G278S and R356H association (K) to C3b was decreased, but FHRS R356H dissociation (k) from C3b was slower, while that of G278S, was faster than the wild type FHRS. Similar results were obtained by ELISA, indicating weaker C3b binding by FHRS G278S. Epitope mapping revealed that C3b binding to FHRS peptides with K144N, V71OM, N718S was increased and binding of C3P to the R356H and M514R mutant peptides was decreased compared to wild type peptides. Altogether, our results identify amino acids within FHRS involved in binding C3b and CRP, and reveal altered ligand binding by some mutant FHRS proteins.
POSTER PRESENTATIONS

P.A6.02.04

Natural killer cell involvement in the development of T cell tolerance

S. Giampaolo, S. Klein-Hessling, F. Berberich-Siebelt, E. Serfling, A. Patra*

1Institute of Pathology and Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany, 2Institute of Translational and Stratified Medicine, Peninsula Schools of Medicine and Dentistry, University of Plymouth, United Kingdom.

In lymphoid cells NFA1c, NFA1c2 and NFA1c3 transcription factors are expressed and involved in antigen-receptor signaling. Specifically, NFA1c plays a critical role in thymocyte differentiation and survival. During T cell development in the thymus, the CD4 CD8 double-negative (DN) cell differentiate to the CD4 CD8 double-positive (DP) stage where they undergo the process of positive- and negative- selection to finally give rise to the CD4 or CD8 single positive (SP) T cells. Based on the expression of CD25 and CD44 molecules, the DN thymocytes again consist of four distinct populations: CD44^+CD25^+DN1, CD44^+CD25^+DN2, CD44^+CD25^DN3 and the CD44^+CD25^DN4 cells. The DN1-3N3 cells are critically dependent on IL-7 signaling for their survival and differentiation. We have shown previously that IL-7 signaling activates NFA1c in the preTCR-negative DN thymocytes in a Jak3-dependent manner leading to its nuclear translocation. Survival of DN thymocytes is partly due to an NFA1c-mediated transcriptional upregulation of the pro-survival molecule Bcl3 in these cells. In vivo binding studies by CHIP-Seq analysis of thymocytes confirmed the binding of NFA1c1 to the Bcl3 gene locus. The indispensability of NFA1c activity was evident as a hematopoietic cell-specific ablation of NFA1c activity resulted in an arrest of thymocyte differentiation at the DN1 stage leading to lymphopenia. On the other hand, overexpression of a constitutively active NFA1c resulted in an impaired migration of DN3 to the DN4 stage, again leading to lymphopenia. These observations suggest that a threshold level of NFA1c activity is critical for efficient T cell development.

José Maria Leukämie - Stiftung

P.A6.02.05

Chronic mucocutaneous candidiasis associated with a rare molecular defect: TRAF3IP2 mutation

N. E. Karaca, A. Aykut, E. Panayat, A. Durmaz, O. Cogulu, G. Aksu, N. Kutukculer*

Ege University Medical School, Izmir, Turkey.

Chronic mucocutaneous candidiasis (CMC) is a recurrent or persistent infections of skin, mucous membranes or nails with Candida albicans and sometimes staphylococcal infections. Patients with autosomal dominant (AD) Hyper-IgE syndrome (STAT3 deficiency) and STAT1 gain-of-function mutation, AR deficiencies in IL-12RB1, IL-12p40, CARD9 or APAPCD (autoimmune-polendocrinopathy-candidiasis-ectodermal dystrophy) syndrome develop CMC as a major infectious phenotype. IL-17 receptor A/F and ACT1 defects in the IL-17 signaling pathway also lead to CMC. A 19-month-old girl, born to second-degree consanguineous parents, referred with recurrent oral thrush and skin eruptions. Elder brother had also CMC. Physical examination revealed oral mucosal candidiasis and multiple purulent eruptions all over the body. Hyperimmunoglobulinemia was observed. Absolute neutrophil counts, lymphocyte subgroups, IgE levels and oxidative burst activity were normal. Candida albicans grew in the mouth swab culture positive for the wound swab. A homozygous c.1560G>C (p.Trp523Cys) mutation was detected in the TRAF3IP2 gene with the targeted next generation sequencing-based Ion AmpliSeq™ Primer ImmuneDiversityPanel. The parents were shown to carry the same mutation as heterozygous. All complaints were recovered by the treatment with trimetoprim-sulfometaxazole and fluconazole prophylaxis.

TRAF3IP2 (TNF-Receptor-Associated-Factor3-Interacting-Protein2) encodes the Act1 molecule, an adapter protein with ubiquitin-ligase activity that binds the IL-17receptor to activate the NFATc1 pathway. This pathway plays a critical role in the control of Th17 and also may be involved in CMC development. A homozygous mutation in TRAF3IP2 would lead to a lack of Act1 protein, which is a crucial component of the NFATc1 pathway.

P.A6.02.06

Overproduction of XBP1s protects from ER stress induced apoptosis in cystic fibrosis primary monocytes

S. Lara Reyna, T. Scambler, A. Aykut, E. Parıltay, A. Durmaz, O. Cogulu, G. Aksu, N. Kutukculer*

1University of Leeds, Leeds, United Kingdom, 2University of Lausanne, Lausanne, Switzerland.

The cystic fibrosis transmembrane regulator (CFTR) is a transmembrane protein, involved in the transport of bicarbonate and chloride ions. Mutation of the CFTR causes cystic fibrosis (CF), resulting in recurrent pulmonary infections and autoimmunization mainly in the lungs. The CFTR is assembled and modified in the endoplasmic reticulum (ER) and, when mutated, accumulation of the CFTR leads to activation of the unfolded protein response (UPR). The UPR comprises three ER transmembrane proteins, known as PERK, IRE1, and ATF6. Chronic UPR activation, through its PERK arm, is linked with apoptosis through accumulation of CHOP. PERK activation can be inhibited by p58IPK production, which is induced by IRE1 activation through XBP1s. The aim of this study was to investigate UPR activation in CF patients. Patients’ peripheral blood mononuclear cells (PBMCs), primary monocytes, and human bronchial epithelial cells (HBEC), were used to evaluate UPR activation, using qPCR and flow-cytometry. LPS, tunicamycin and thapsigargin were used as cellular UPR stimulants to assess UPR activation.

Gene expression revealed a significant increase in XBP1s in HBEC lines, PBMCs, and monocytes from CF patients. IRE1a protein expression was also increased in the three CF cell lines. Furthermore, CF monocytes pre-treated with the IRE1 inhibitor, 4μBC, showed a significant increase in the activity of PERK, including AT4, GADD34, and CHOP, after UPR activation. Finally, PERK overactivation correlated with the downregulation of p58IPK in CF monocytes after 4μBC pre-treatment.

Data suggest that misfolded CFTR proteins induce ER stress in CF. Furthermore, XBP1s overproduction protects CF cells from CHOP-induced apoptosis.

P.A6.02.08

Linker for activation of T cells (LAT) inhibits development of aggressive thymic lymphomas by downregulating Notch-1 and -p7a expression

K. Marek-Bukowiec, A. Aykut, E. Parıltay, A. Durmaz, O. Cogulu, G. Aksu, N. Kutukculer*

1Centre d’immunologie de Marseille Luminy, Marseille, France, 2Institute of Translational and Stratified Medicine, Peninsula Schools of Medicine and Dentistry, University of Plymouth, Plymouth, United Kingdom.

The cystic fibrosis transmembrane regulator (CFTR) acts as a thymocyte development checkpoint. Thymocyte development is arrested at the CD4− CD8− CD25+ (DN3) stage but this block can be relieved when these mice are crossed with a transgenic line bearing a LAT∥-/- line. The LAT∥-/- line overexpresses a constitutively active NFATc1 resulted in an impaired transition of DN3 cells to the DN4 stage, again leading to lymphopenia. These observations suggest that the first ACT1 deficiency patient after two siblings who were diagnosed in 2013 by Boisson B et al.

P.A6.02.09

Cellular disease in patients from Cantabria (northern Spain): distribution of risk HLA haplotypes


Celiac disease is one of the most prevalent genetically determined autoimmune diseases, and one of the diseases with strongest association with particular HLA haplotypes, specifically with DQ2 and DQ8. Regarding the risk of the HLA haplotypes, several discrepancies among populations have been found, mainly due to not take into account the difference between the HLA DQ2.2 and DQ2.5 molecules.

For this reason, we genotyped 781 celiac disease patients (492 children < 15 years old, and 289 adults) who fulfilled the ESPGHAN criteria for this disease by using PCR-SSO in a Luminesix platform.

In our population, when we analyzed together children and adults, we found some differences in the frequency of the haplotypes associated with an increased susceptibility to celiac disease compared with previously reported populations, and in the risk that these haplotypes conferred to the disease, mainly in the distribution of DQ2.2/DQ2.2, DQ2.2/x, DQ2.2/DQ8 and DQ8 either in homocigosity or heterocigosity. Interestingly, half of DQ2.2/x carried the DQA1*04 in trans position. We think that the main reasons of these differences are: a) the different prevalence of these haplotypes among populations and b) lack of separate analysis between DQ2.2 and DQ2.5.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 209
This study is the first applying NGS on genetically well-defined human B cell samples and establishes the 5 pathway model for the mechanism of SHM based on human data. We performed next generation sequencing of the B-cell receptor heavy chain locus in a unique group of patients with bi-allelic mutations in genes involved in BER (DNA repair). The generation of high affinity antibodies is dependent on somatic hypermutation (SHM). SHM is initiated by the activation induced cytosine deaminase (AID) which generates U:G to T:A transversions. We found a remarkable correlation of alleles and genotypes at rs1310182 as follow T allele (OR = 0.33 - 0.83, 95 % CI = 0.53, P <0.01), C allele (OR = 1.19 - 2.19, 95 % CI = 1.37 - 2.18, P < 0.01), TT genotype (OR = 0.39 - 0.94, 95 % CI = 0.43, P = 0.03) and GG genotype (OR = 1.37 - 4.76, 95 % CI = 2.32, P = 0.01) in UC. This association study showed that rs1310182 SNP of PTPN22 could have role in UC; however, more researches on this topic are needed. Hidradenitis suppurativa (HS) is a chronic, inflammatory skin disease of the hair follicle characterized by relapsing painful inflammatory nodules, abscesses and fistula tracts in the apocrine gland-bearing areas of the body, most commonly in the axillae, inguinal and perineal regions. To date, no study has looked for the role of the killer cell immunoglobulin-like receptors genes (KIR) in the pathogenesis of this disease. KIR, found on the surface of natural killer (NK) cells, play a key role in controlling the innate response. To study the role of the presence / absence of KIR genes in the pathogenesis of HS. In the same way, KIR haplotype and genotype distribution is analysed. A total of 106 patients with HS and 262 age and sex-matched healthy controls were studied for the presence / absence of KIR genes by PCR-SSO and Luminescence analysis. We only found a weak difference in the distribution of KIR genes in patients with healthy controls. Only KIR2DL3 was found to be less frequent in HS patients vs healthy controls (79% vs 89.8%, p = 0.13 OR 0.49 CI95% 0.22-0.81) suggesting a protective role against HS. This is the first study trying to find an association of KIR genes with HS. Our results suggest that KIR genes do not influence in a significant way on resistance/susceptibility to HS.
Whole exome sequencing disclosed heterogeneous gene defects in pediatric SLE

S. Zoghbi\textsuperscript{1}, P. V. Ziae\textsuperscript{1}, E. Salam\textsuperscript{2}, T. Hirschmu\textsuperscript{2}, R. Jimenez-Verdol\textsuperscript{1}, A. Krolo\textsuperscript{1}, K. Bautz\textsuperscript{1,3}, N. Rezaei\textsuperscript{1,2,4}.

\textsuperscript{1}Department of Immunology, School of Medicine, University of Medical Sciences, Tehran, Iran, Islamic Republic of; \textsuperscript{2}Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of; \textsuperscript{3}Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna, Austria; \textsuperscript{4}Division of Pediatric Rheumatology, Children’s Medical Center, Pediatrics Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of.

This study not only provides fundamental insights into how CD37 organises the B cell membrane, but also contributes to the development of new immunotherapies for patients suffering from aggressive B cell lymphoma. Indeed, we have shown that lymphoma cell lines lacking CD37 often also lack CD20 expression. Importantly, these B cell lines are less responsive to Rituximab treatment when compared to CD37-positive counterparts.

Immune cell function is heavily dependent on the proper localisation of many surface molecules. This localisation is for a large part orchestrated by tetraspanin proteins. This receptor blocking. All these data offer a new approach to the improvement of tumor immunotherapy by the combination with metabolic inhibitors.

Materials and Methods: Several leukemic or multiple myeloma cell lines were supplemented for 72h with non toxic concentrations of DCA or of metformin, and then they were stimulated to proliferation. DCA, an inhibitor of pyruvate dehydrogenase kinase, forces cells to obtain energy through mitochondrial oxidative phosphorylation and is used in the treatment of lactic acidosis. Metformin is the most common treatment of type II diabetes, and decreases glucose concentration in blood. It inhibits mitochondrial complex I, but has pleiotropic effects, e.g., activation of AMPK.

Introduction: Dichloroacetate (DCA), an inhibitor of pyruvate dehydrogenase kinase, forces cells to obtain energy through mitochondrial oxidative phosphorylation and is used in the treatment of lactic acidosis. Metformin is the most common treatment of type II diabetes, and decreases glucose concentration in blood. It inhibits mitochondrial complex I, but has pleiotropic effects, e.g., activation of AMPK.

Histone-deacetylases (HDACs) are a group of enzymes that control histone/non-histone deacetylation. Certain members of HDAC family control the function of macrophages. We aimed to study the expression of HDACs in macrophages isolated from inflammatory bowel diseases (IBD) patients.

Macrosкопially inflamed and non-inflamed colon resection tissue were collected from 15 Crohn’s disease (CD) and 9 ulcerative colitis (UC) patients operated on for therapy refractory disease. Lamina propria was separated from the muscularis externa, and a targeted array for epigenetic enzymes was performed.

From our array, gene expression of HDAC9 in inflamed macrophages from CD was decreased compared to non-inflamed macrophages from UC (p<0.005). In addition, in CD, HDAC9 mRNA level was increased in inflammation in comparison to non-inflamed tissue (p=0.046). To assess the relevance of HDAC9 gene expression in terms of protein level, immunofluorescence staining of HDAC9 protein was undertaken in tissue sections from inflamed and non-inflamed mucosa. CD68 was used as a pan-macrophage marker. In conjunction with the expression data, HDAC9 protein was found highly expressed in inflamed tissue.

Restoration of EROS expression. Further, we describe a case of CGD secondary to a homozygous EROS mutation that abolishes EROS protein expression. This work demonstrates the fundamental importance of EROS in human immunity and describes a novel cause of CGD.

Whole exome sequencing displayed CD37 as a possible new therapeutic target in pediatric SLE.

P.B.01.01 Tumor vaccination principles and Immunotherapy - Part 1

P.B.01.01.01

Dichloroacetate and metformin sensitize human tumor cells to the cytotoxic action of NK cells and CTL

J. Marco-Brussa\textsuperscript{1}, N. Allende-Vega\textsuperscript{1}, O. Gonzalez\textsuperscript{1}, L. Marzo\textsuperscript{1}, M. Villalba\textsuperscript{1}, A. Ane\textsuperscript{2}.

\textsuperscript{1}University of Zaragoza, Zaragoza, Spain, \textsuperscript{2}Institute for Regenerative Medicine and Biotechnology (IRMB), Montpellier, France.

Introduction: Dichloroacetate (DCA), an inhibitor of pyruvate dehydrogenase kinase, forces cells to obtain energy through mitochondrial oxidative phosphorylation and is used in the treatment of lactic acidosis. Metformin is the most common treatment of type II diabetes, and decreases glucose concentration in blood. It inhibits mitochondrial complex I, but has pleiotropic effects, e.g., activation of AMPK.

Materials and Methods: Several leukemic or multiple myeloma cell lines were supplemented for 72h with non toxic concentrations of DCA or of metformin, and then they were used in cytotoxic assays with expanded HDAC9 as a positive control of HDAC9 expression.

Results: DCA sensitizes the human multiple myeloma MM1S to cytotoxicity exerted by NK cells and by CTL. The combined blocking of NKGD2 ligands and LFA-1 regulates DCA sensitization to CTL without affecting basal cytotoxicity. LFA-1 blocking abrogates NK cell cytotoxicity on MM1.S cells and, in consequence, also DCA sensitization. On the other hand, metformin sensitizes the human B-CLL cell line Mec1 to CTL and especially to NK cells.

This sensitization is also observed in Mec1 cells overexpressing the anti- apoptotic proteins Bcl-x\textsubscript{L}, LFA-1 blocking, but not NKGD2 ligand blocking, abrogates the metformin-induced sensitization to NK cells observed on Mec1 and on Mec1-Bcl\textsubscript{L} cells. Finally, metformin sensitization to NK cell cytotoxicity on Mec1-Bcl\textsubscript{L} cells is partially prevented by death receptor blocking. All these data offer a new approach to the improvement of tumor immunotherapy by the combination with metabolic inhibitors.

The influence of tetrassin CD37 on the expression and distribution of CD20 on lymphoma B cells


Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, Netherlands.

Immune cell function is heavily dependent on the proper localisation of many surface molecules. This localisation is for a large part orchestrated by tetrassin proteins. This superfamily of 4-transmembrane proteins forms protein clusters to ensure proper distribution of surface receptors needed for immune cell interactions. Tetrassin CD37 is exclusively expressed by immune cells, but has its highest expression on B cells. Recent findings of our group show a critical role for CD37 expression in the clinical outcome of patients suffering from aggressive B cell lymphoma.

The standard treatment for B cell lymphoma is a combination therapy using chemotherapy (CHOP) and the anti-CD20 antibody Rituximab. Patients with CD37-negative lymphoma respond less to this combination therapy, indicating that CD37 controls expression and/or distribution of CD20 on the cell surface. This is supported by preliminary data showing that lymphoma cell lines lacking CD37 often also lack CD20 expression. Importantly, these B cell lines are less responsive to Rituximab treatment when compared to CD37-positive lymphoma cell lines.

We hypothesise to elucidate the role of CD37 in the expression, distribution and localisation of CD20 on healthy B cells, lymphoma cell lines and patient lymphoma cells. We established an extensive array of aggressive B cell lymphoma cell models in which CD37 expression is manipulated by either overexpression or complete knock-out using the CRISPR/Cas9 technique. This study not only provides fundamental insights into how CD37 organises the B cell membrane, but also contributes to the development of new immunotherapies for patients with aggressive B cell lymphoma.
A. Kunert, H. Abken; 1Hospital Saint Louis, Paris, France, 2INSERM U970, Paris, France.

Introduction: Synthetic melanin bound to subunit vaccine antigens enhances CD8+ T-cell responses in mice, when combined with a TLR9 agonist. We here compared the efficacy of various vaccine adjuvants, alone or in combination with melanin, to trigger CD T-cell responses. Material and Methods: Two peptides containing a gp100 or an ovalbumin epitope were mixed with L-Dopa and further oxidized to generate melanin-bound peptides. Different TLR9 agonists (Cpg-28, 1826, IS3), polyinosinic-polycytidylic acid (Poly-IC), Freund adjuvant (Alum) or liposomes (Lipo) were mixed with melanin to generate vaccine formulations. The mice were immunized subcutaneously (n=18/group), and the CD8 immune response was assessed with epitope-specific IFNγ production by splenocytes. Results: When Alum was used as an adjuvant, no significant CD8 response was seen, even with melanin-bound peptides. With Freund adjuvant, combined or not with a TLR9 agonist, a mild CD8 response was seen using either free or melanin-bound peptides. On the contrary, both TLR9 agonists and poly-IC elicited a CD8 T-cell response with free peptides, and this response was several fold enhanced when melanin-bound peptides were used, mostly when the amount of melanin was increased. The cells showed an effector memory CD8 T-cell phenotype. The minimum dose of peptides required to trigger immunity was 0.5 µg. Immunization against the ovalbumin epitope inhibited the growth of ovalbumin-positive tumors. Conclusion: The conjugation of melanin to peptides represents a very simple means of triggering CD8 T-cell response, which should be particularly useful in cancer immunotherapy against neo-epitopes.

P.B1.01.04

Immune response induced in mice by co-delivery of STING recombinant and HPV DNA vaccine

T. Tran, E. Tartour, A. F. Carpenter2

+Clinical Laboratory, Linnaeus People’s Hospital, Hongkou, China, Institute of Immunology, School of Medicine, Zhejiang University, Hangzhou, China.

Cervical cancer is the second most common cancer among women worldwide and remains a clinical problem despite improvements in early detection and therapy. HPV DNA vaccines have become an attractive approach to treat HPV-related cancer. To investigate the effect of STING in immune response induced by plasmid encoding HEV67 epitope and to explore new strategies for prophylactic and therapeutic HPV DNA vaccines, C57BL/6 mice and TC-1 tumor cell bearing mice were immunized with pVAX-HEV67 alone or co-immunized with pVAX-STING. The specific CTL response and Th1 cell response were also assayed. The co-immunization of pVAX-STING and pVAX-HEV67 significantly inhibited the growth of TC-1 after immunization and prevent the TC-1 tumor cell bearing mice from developing into tumor compared with the mice immunized with pVAX-HEV67 alone. Furthermore, the immunological mechanism behind immune enhancing effect of STING co-immunization was in accordance with the up-regulation of Th1 cytokine IFN-γ. The results showed that co-administration of STING could elicited stronger immune response induced by HPV DNA vaccines and it provided scientific basis for the further use of STING as a HPV DNA vaccine adjuvant. This work was supported by grants from Zhejiang Provincial Natural Science Foundation of China (No. LY14H000003), Science and Technology Department of Zhejiang province(No.2016C371211) and Health and Family Planning Commission of Zhejiang province(No.2016KYB02).

P.B1.01.05

Comparing the efficiency of two clinical grade stimuli on BDCA3 mDCs by using transcriptomics

T. S. Mathan1, G. Fórez-Grau1, T. van Oorschot1, S. J. Busch1, G. Schreiber1, I. Reinierinen-Beerens2, D. Sanchez1, C. Alford1-4, C. G. Figdor1, L. M. de Vries1, J. Testor1

1Department of Tumor Immuno-Research, Erasmus Medical Center, Rotterdam, Netherlands, 2Department of Molecular Oncology, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands, 3Department of Gastroenterology and Hepatology, Erasmus MC-University Medical Center, Rotterdam, Netherlands, 4Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain, 5Division of Gene Therapy and Hepatology, Centre for Applied Medical Research (CIMA, Pamplona) Spain, 6Department of Oncology, University Clinic of Navarra, Pamplona, Spain, 7Department of Immunology, University Clinic of Navarra, Pamplona, Spain, 8Department of Medical Oncology, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands.

Maturation of dendritic cells (DC) is considered critical in cancer immunotherapy. Among different human subsets, BDCA-3 (CD141-high Clec9A+) DCs are attracted and recruited to tumors, where they acquire an activated and migratory phenotype. Using a novel transgenic mouse model expressing MSF, an angiogenic fibronectin domain, we found that MSF was induced in tumor-associated macrophages (TAMs) and CD8+ T cells in tumors. This up-regulation was confirmed by immunohistochemistry and immunofluorescence in human tumour cells and TAMs. Double immunostaining indicated that most of the CD68, CD206 and CD163 positive TAMs and CD8+ T cells co-localized with MSF. This suggests that MSF is selectively associated with the M2 polarization of macrophages. We developed original reagents (recombinant protein, monoclonal antibodies) for MSF study. MSF production was assessed in co-culture of fetal fibroblasts and tumor cells. Here we report that MSF was induced mostly by M-CSF, IL-4 and IL-10 but not by proinflammatory stimuli. RNA analysis clearly demonstrated that it was induced in TAMs and tumor cells. These findings suggest that MSF may facilitate monocyte extravasation and migration, a critical step for the development of the tumor microenvironment.
POSTER PRESENTATIONS

Treatment with TCR-iL12 but not iL18-T cells resulted in enhanced intra-tumoral accumulation of macrophages, accompanied by a decreased frequency of therapeutic CD8+ T cells. This observation, when administered to mice, iL18 but not iL12 demonstrated a favorable profile of T cell co-stimulatory and inhibitory receptors. In conclusion, we observed that treatment with T cells engineered with a TCR and iL18-T cells is safe and able to skew the tumor microenvironment in favor of an improved anti-tumor T cell response.

P.B1.01.10

IL-12-dependent Th1 priming by a DC vaccine in vivo requires cooperation of dendritic cells subsets

D. Ashour, M. B. Lutz
University of Würzburg, Würzburg, Germany.

The production of heterodimeric IL-12p70 by injected vaccine dendritic cell (DCs) has been classically described as a key factor required for generating polarized T helper type 1 cell response (Th1). However, cocktail-matured DCs do not secrete IL-12 but readily induce Th1 responses when injected into mice and humans. Here, we tested for DC-DC cooperation enabling bystander IL-12 production for Th1 polarization in a DC vaccination model. Subsequently injected iLPS-matured and OVA peptide-loaded bone marrow-derived DCs (BM-DCs) migrated to the draining lymph node in the recipient mice and induced responder CD4+ OT-II T cells to polarize into Th1 cells. Injected CCR7/- DCs, which lack migratory capacity, failed to induce OT-II cell priming and Th1 induction, indicating that the injected DCs provide the peptide presentation and costimulation. DC vaccination increased also the migration of all endogenous skin-resident DC subsets to the draining lymph nodes. However, only the CD103+ dermal DCs showed increased IL-12p40/FcγII production culminating at 72h after BM-DC injection. Surprisingly, injections of BM-DCs derived from p35-/- mice did not affect Th1 priming, while deficiency of p35 in the recipient mice abrogated the IL-12-dependent Th1 priming, which indicates that IL-12 production by endogenous DCs and not the injected BM-DCs is required for Th1 priming in this DC vaccine model in the draining lymph node. Further studies will show the requirement of IL-12 from CD103+ DCs for Th1 priming. Together, our data indicate that DC vaccines require a cooperation with endogenous DCs for optimal Th1 polarization of CD4+ T cells.

P.B1.01.11

Gene-edited chimeric antigen receptor (CAR) T cells: Tuning up for the next generation cancer immunotherapy

H. R. Miraæ, F. Raghani, H. Miraæ
1Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of; 2Ishfahan University of Medical Sciences, Isfahan, Iran, Islamic Republic of.

Recently clinical trials utilizing genetically engineered T cells expressing a chimeric antigen receptor (CAR) that is half monoclonal antibody and half T-cell receptor have demonstrated remarkable response in patients with advanced cancers like relapsed or refractory acute lymphoblastic leukemia (ALL) and lymphoma. Moreover, emerging chimeric genome editing tools such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas composed of sequence-specific DNA binding module(s) linked to a non-specific DNA cleavage domain have made possible to dramatically expand the ability to manipulate cells aim to treat and/or study a wide range of diseases including cancer. Here, we will discuss how joint application of these two chimeras will help us to manipulate CAR T cells aiming to enhance the efficacy of CAR T cell therapy in preclinical and clinical settings.

P.B1.01.12

Effects of ionizing radiations on T and B lymphocytes: a comparison between photons and protons

F. Novelli1, M. Vadrucchi1, M. M. Rosado1, L. Picardi1, C. Ronisvall1, E. Benvenuto4, C. Marinò1, C. Piaf4.1 1ENEA, Division of Health Protection Technologies, Rome, Italy, 1ENEA, Laboratory of Development of Particle Accelerators and Medical Applications, Rome, Italy, 1Freelance Research Centre for Advanced Microscopy, Rome, Italy, 1ENEA, Laboratory of Biotechnology, Rome, Italy.

Photon and proton therapy (y/n-rays) is widely used to treat a large variety of cancers, while particle-based radiotherapy (protons or carbon ions) represents a developing and valuable option, especially for cancers requiring more focused treatments. Indeed, proton beams display low entrance dose, uniform high dose on targeted tumor (spread-out-bragg peak) and near zero dose beyond it, thus preserving not targeted tissues. Recent studies indicated that photon and particle radiations produce different biological effects, with relevant outcomes combined as radio-immune therapies. We compared the effects of low in vivo exposure (2 Gy) to medium energy proton and X-ray beams on mouse lymphoid spleen cells. Proton irradiation was carried out with the 27 MeV beam produced by the TOP-IMPLANT pulsed linear accelerator. For X-rays, a CHF320G generator (250 kV, 15 mA) was used. During the exposure, mice (C57Bl/6) were anesthetized; not targeted areas were protected by shields. At different time points after irradiation (1-28 days), mice were analyzed for number and phenotype of different T (CD4/CD8+) and B lymphocytes, as well as for the number of splenic macrophages. Results: The babassu mesocarp extraction (BME) yield was 0.25±0.01% dry weight; the total sugar amount was 29.79 mg/ml containing monosaccharides, reducing sugars, polysaccharides and 0.506 mg/ml total protein. Chromatography in silico showed that BME sensitization with cancer cells modulated an immune response in Balb/c animals, indicating an immunogenic effect. Key words: Arecaceae, Attalea speciosa, Th1/Th2 response, T helper and B lymphocytes.

P.B1.01.13

Activation of murine splenocytes against tumor cells by sensitization with babassu mesocarp


Introduction: Attalea speciosa Mart. (babassu) fruit contains a mesocarp that is rich in carbohydrates with immunomodulatory effects. The induction of the tolerogenic response is a tumor escape mechanism, and immunosuppressive mechanisms have been shown to reestablish host immunosuppression. This study evaluates the adjuvant potential of babassu mesocarp carbohydrates in a tumor model. Materials and Methods: The polysaccharide obtained from aqueous babassu extract (20 mg/ml) was used to sensitize the animals inoculated or not with the tumor to obtain splenocyte for the phenotypic characterization and lymphoproliferation assay. Results: The babassu mesocarp extraction (BME) yield was 75.54%, and the total sugar concentration was 29.79 mg/ml containing monosaccharides, reducing sugars, polysaccharides and 0.506 mg/ml total protein. Chromatography identified glycosides, glycosides and fructose. Sensitization increased the spleen weight in the tumor group compared with the control, and a comparatively lower frequency of T helper and higher frequency of B-lymphocytes was also observed. Conclusion: These results showed that local exposure to X-rays or protons induced changes in many of the analyzed parameters, with different effects and recovery time depending on the type of radiation used.

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P.B1.01.14

HPMA copolymer-bound doxorubicin as an endogenous vaccine substantially increases the therapeutic effects of check-point blockade monoclonal antibodies

B. Rihova, T. Etrych1, R. Stepenková, V. Šuber, K. Uličnich, M. Kovar, M. Sirova2.1Institute of Microbiology AS CR, Prague, Czech Republic, 2Institute of Microbiology AS CR, Prague 6, Czech Republic, 3Institute of Microbiology AS CR, Prague 4, Czech Republic

DOXii-pHPMA is a doxorubicin bound to a synthetic polymeric carrier based on N-2-hydroxypropyl)methacrylicamide via a pH-sensitive hydrazide bond. It is effective anticancer polymeric prodrug with decreased side-effectivity and the ability to induce immunogenic cancer cell death releasing tumor antigens and thus acting as endogenous vaccine. We investigated a novel combination strategy using low dose of DOXii-pHPMA and immune checkpoint blocking anti-CTLA-4 and anti-PD-1 mAbs to treat EJ T cell lymphoma and 4T1 breast carcinoma. Acute model of disease when mice are transplanted once with a lethal dose of tumor cells was compared with chronic model where mice are injected six times every other day with a low number of tumor cells. Significant toxicity of anti-CTLA-4 and anti-PD-1 mAbs was seen in mice suffering from acute disease. Mice with chronic cancer respond to treatment much better than those with acute disease. Therapy with anti-CTLA-4 and/or with anti-PD-1 only led to twenty-five percent survival of the treated mice, which were considered long-term survivors (LTS). On the other hand mice more than 60% of mice injected also with therapeutically suboptimal dose of DOXii-pHPMA survived disease-free for more than 100 days. In germ-free mice, tumor growth was more aggressive, and the survival time was shorter. Treatment with DOXii-pHPMA showed only limited anti-cancer effects while the combination with the anti-CTLA-4 monoclonal antibody significantly improved the therapeutic outcome. The best results were seen in germ-free mice monoclonalized with B. thetaiotaomicron. This work was supported by the Ministry of Health CR (grant number 16-28600A) and the CSF (grant number 17-08048S).
POSTER PRESENTATIONS

P.B1.01.15
PD-L1 expression according to five monoclonal antibodies in uterine cell cancer: concordance and clinical implications
M. Rijnders1, A. van der Veldt2, J. Boormans3, E. Zwarthoff4, M. Lolkema5, R. de Wit6, A. van Leenders7; 1Erasmus MC, Rotterdam, Netherlands, 2Radiodiagnostics, Nijmegen, Netherlands, 3VUMc, Amsterdam, Netherlands.

Introduction: High PD-L1 expression in uterine cell cancer (UC) shows conflicting results, which may be confounded by the use of different PD-L1 companion diagnostics. The objective of this study was to accurately compare PD-L1 expression of five commercially available PD-L1 antibodies in UC patients. Methods: Tissue Microarrays (TMA) containing samples of 141 mucinous UC patients (52%) were stained with the anti-PD-L1 antibodies 22C3, 28-8, SP142, SP263 and E1L3N on the Ventana Benchmark (SP142, SP263) and DAKO platforms (22C3, 28-8, E1L3N). PD-L1 expression was manually scored on tumor cells and infiltrating immune cells according to corresponding assay specifications used in clinical trials. Results: PD-L1 expression was found to be positive in 20% (SP263), 21% (SP142), 23% (28-8), 27% (22C3), and 27% (E1L3N) of cases. No relations between clinicopathological parameters and PD-L1 expression were observed. Concordance in treatment-determining score varied from 72% to 90% and was lowest for E1L3N (mean 75%). Considering only companion diagnostics tests 22C3, 28-8, SP142 and SP263, PD-L1 status was concordant in 78% of patients. When one test result was discordant (n=15), 13% (SP142) and 28% (n=5) were most likely different. Conclusion: Agreement of PD-L1 assessment is good with similar PD-L1 status by four antibodies used in companion diagnostic tests. Therefore, application of different companion PD-L1 antibodies and platforms may have limited effects on therapeutic decision making in IC therapy.

P.B1.01.16
Oncolytic adenovirus coding for TNFa and IL-2 removes the need for lymphodepleting preconditioning in adoptive T-cell therapy
J. Santasi1, V. Cervero-Carrascon1, R. Havunen2,3, M. Siurala4, S. Sorsa5, M. Anttila6, A. Hemminki7,8; 1TILT Biotechertres Ltd, Helsinki, Finland, 2Cancer Gene Therapy Group, Helsinki, Finland, 3Finnish Food Safety Authority (EVIRA), Helsinki, Finland, 4Helsinki University Hospital Cancer Centre, Helsinki, Finland.

Introduction: Lymphodepleting preconditioning with high-dose chemotherapy remains a critical component for the clinical effectiveness of several adoptive T-cell therapy (ACT) strategies. This preconditioning step boosts the antitumor efficacy of transferred T cells through the decrease of tumor immunosuppression, however, with severe toxicity for patients. In contrast, oncolytic adenoviruses are safe and when engineered to express interleukin-2 (IL-2) and tumor necrosis factor alpha (TNFa), they can achieve immunomodulatory effects similar to lymphodepleting preconditioning.

Materials and Methods: Here, we compare the safety and efficacy of such oncolysius with a cyclophosphamide and fludarabine lymphodepleting regimen in the context of ACT. Since Syrian hamsters allow replication of a human adenovirus (Ad5/3-E2F-244 TNFa-IRES-hTERT), we used a pancreatic tumor model (HapT1) in syngeneic hamsters infected with tumor infiltrating lymphocytes (TIL). To study immune cells responses to TNFa and IL-2, we used an immunocompetent mouse melanoma model (B16.OVA) infected with an adenovirus expressing IL-2 and TNFa.

Results: Animals receiving oncolytic adenovirus therapy demonstrated better tumor growth control and survival compared with those receiving lymphodepleting chemotherapy. Moreover, the adenovirus approach increased the levels of TNF-a cytokines and infiltration CD8+ T cells and CD11c+CD86+ dendritic cells. While lymphodepleting preconditioning resulted in severe toxicities in the heart and lungs, adenovirus therapy caused minimal changes in treated animals.

Conclusion: Overall, this data shows that ACT protocols using oncolytic adenovirus expressing IL-2 and TNFa do not require high-dose preconditioning chemotherapy. Clinical translation is ongoing in a Phase I clinical trial where melanoma patients administered with TIL therapy receive HAP-T123 instead of lymphodepleting chemotherapy.

P.B1.01.17
Tumor-type-specific spatio-temporal shifts of lymphoid and myeloid populations during tumor growth and checkpoint blockade
S. T. T. Schetters1, L. J. Kruissien, M. H. Crommentuut, V. Van Kooyk; VU University Medical Center, Amsterdam, Netherlands.

Suppression of the immune system by solid malignancies has proven to be a driving force of tumor development and an effective target for therapeutic intervention. Especially the suppression of cytotoxic T cells through inhibitory receptors, like PD-1 and CTLA-4, can be blocked by antagonistic antibodies, reinvigorating existing anti-tumor responses. However, it unclear whether immune checkpoint interactions are heterogeneous within the tumor, how these interactions develop during tumor growth and which cell types interact in the tumor microenvironment. By using high dimensional flow cytometry and unsupervised clustering analyses based on immune checkpoints, we show heterogeneity of tumor-infiltrating CD8+ and CD4+ T cells in murine B16 melanoma and MC38 colorectal carcinoma. Also, we show that the myeloid- and tumor cell compartments provide the ligands for immune checkpoint suppression. Next, we show that a therapeutic intervention using anti-PD1 treatment, changes lymphoid and myeloid populations and existing immune checkpoints interactions. Finally, we reconstruct the the tumor microenvironment using 8-color confocal microscopy and histocytometry analysis to spatially reconstruct the changing immune compartments of the tumor microenvironment.

P.B1.01.18
Human Monoclonal IgGs derived from patients with Multiple Myeloma are able to penetrate living neoplastic cells and induce apoptosis
T. Stivarou1, J. Sarrigeorgiou2, P. Cholai1, A. Tsiragianis3, P. Lymberi1, C. Tsalgouz1; 1Immunology Laboratory, Immunology Dept. Hellenic Pasteur Institute, Athens, Greece, 2Immunology-Histocompatibility Dept. Evagelismos General Hospital, Athens, Greece.

Introduction: Antibodies able to penetrate living cells (CPAbs) have been well characterized in patients with SLE and in mouse models. Our lab has described the existence of CPAbs with high-affinity to the neoplastic and the normal immunoglobulin (Ig). The development of human monoclonal IgGs (c-CPAbs) is of major importance in drug delivery and in cancer immunotherapy. The aim of this preliminary study was to identify such mAbs among serum monoclonal immunoglobulins (M-IgG) from patients with Multiple Myeloma (MM), and further study their intracellular biological functions in neoplastic cells.

Methods: 71 sera from IgG-MM patients were studied by in-house ELISAs against 7 self & non-self antigens. We purified 6 IgG, 5 polyreactive & 1 non-polyreactive by protein-G affinity chromatography and tested them in optimum conditions for ability to: 1) penetrate FcγR+ (Raji & MDA-MB-231) and FcγR- (NIH-3T3 & HeLa) cells by immunofluorescence, 2) induce apoptosis on these cells by flow cytometry, and 3) hydrolyze plasmid & genomic DNA.

Results: The 5 purified M-IgGs tested were able to penetrate all cells, either FcYR+ or FcYR-, at 37°C in a dose- and time-dependent mode of entry, while 3/5 also penetrated cells at 4oC (energy-independent entry). All M-IgGs were polyreactive, accumulated in the cytoplasm, induced apoptosis especially in MDA-MB-231 cells, and hydrolyzed plasmid DNA.

Conclusion: The IgG-MM sera represent an excellent source of human mIgGs exhibiting cell-penetrating ability and intracellular functionality, and can be exploited as a potential therapeutic tool, used either per se, or as carriers for intracellular drug delivery, or even both.

P.B1.01.19
Highly specific targeting of human acute myeloid leukemia (AML) cells using functionalised gold nanoparticles
I. M. Yasinska1, B. F. Gibb2, R. Hussain3, G. Siligardi4, E. Fasler-Kan5, L. Calzolai6, V. V. Sumbayeva6; 1University of Kent, Chatham Maritime, United Kingdom, 2Diamond Light Source, Didcot, United Kingdom, 3University Hospital Bern, Inselspital, Bern, Switzerland, 4European Commission Joint Research Centre, Ispra, Italy, 5University of Kent and Greenwich, Chatham Maritime, United Kingdom.

Highly specific targeting of human malignant cells with the purpose of recognition and delivery of specific drugs into them is a very promising but not well developed complex of diagnostic and therapeutic strategies. It is a major focus of current molecular cancer research, Immunology and Nanomedicine. In this study we demonstrated for the first time a new approach for highly specific targeting of human acute myeloid leukemia (AML) cells by functionalised gold nanoparticles carrying single-chain antibody against the immune receptor Tim-3 and rapamycin. Tim-3 is highly expressed in human AML cells. It is one of the key components of Tim-3/galecin-9 secretory pathway which is crucial for survival of myeloid cells because it determines their ability to escape host immune surveillance. Thus, Tim-3 can be used as a target for specific recognition of AML cells. Rapamycin inhibits activity of mammalian target of rapamycin (mTOR), a master regulator of translational pathways in AML cells. Inhibiting the mTOR leads to a rapid killing of AML cells. Using these nanocojngutes we managed to successfully deliver rapamycin into the AML cells reaching attenuation of the mTOR activity. Concentration of rapamycin required to reach such an effect is at least 50 times lower compared to the one of free rapamycin required to achieve similar effect. We therefore conclude that our technology is of potential use for highly specific targeting of AML cells for the purpose of diagnosis and possibly therapy. The nanocojngutes can be used specifically to identify malignant blood cells thus allowing rapid AML diagnosis.
Exosomes mediated protective cancer vaccine: in vivo performance
1Bilkent University-Department of Molecular Biology and Genetics, Ankara, Turkey, 2Immunology and Allergy Unit, Department of Medicine Salina, Karolinska Institute, and Karolinska University Hospital, Stockholm, Sweden, 3Department of Biological Sciences, Middle East Technical University, Ankara, Turkey.

Exosomes are naturally occurring nanovesicles that have attracted considerable attention as drug delivery vehicles. Recently, nanoparticles were explored extensively for drug delivery applications aiming to improve better therapeutic read-outs due to their low toxicity and biocompatibility. Herein, we describe a simple method to externally load magnetic nanoparticles (FeO-NPs) along with TfR ligands within exosomes. The internalization and immunomodulatory activities of unloaded or TfR, TfR ligand and FeO-NP loaded exosomes were analyzed either on RAW264.7 murine macrophage-like cell line or on spleocytes at various time and dose intervals. Furthermore, therapeutic efficacy of exosomes loaded with FeO-NPs and TfR ligands (i.e. Exo(p(CpG+FeO)) were tested on hu-HUH7-bearing athymic mice. Our data showed that FeO-NPs loaded exosome (Exo(FeO)) uptake by RAW264.7 and spleocytes enhanced up to 10-fold and 3-fold, respectively compared to unloaded exosomes. Moreover, co-encapsulation of CpG with FeO-NPs (Exo(p(CpG+FeO))) within exosomes significantly magnified CpGODN internalization by RAW 26.4.7 macrophages compared to FeO-NP devoid CpG ODN loaded exosomes (Exo(p(CpG))). As expected, Exo(p(CpG+FeO)) treated spleocytes secreted higher amounts of IL-2 compared to Exo(p(CpG)). Lastly, mice were xenotransplanted with huH7 cells and palpable tumor formation was formed. Tumor-bearing animals that were treated with Exo(p(CpG+FeO)) showed significant regression compared to the control group. Therefore, we concluded that exosomes loaded with magnetic nanoparticles (FeO-NPs) and TfR ligands within exosomes offers an effective therapeutic strategy for developing targeted exosomes that could boost their therapeutic impact.

P.B1.01.21
Implantable, pre-activated microconed-Si scaffold vaccines for cancer therapy
I. Zerva1, C. Lanara2, E. Stratakis2, I. Athanassakis2
1Department of Biology, University of Crete, Heraklion, Greece, 2FORTH, Heraklion, Greece.

Therapeutic vaccines are an active immunotherapy of cancer selection aimed at the patient’s therapy using immune system of the patient. Over the years, the lack of effective active immunotherapies for cancer has led to the development of many new strategies. One of the major problems is the failure of development of immune responses against tumor antigens since these are usually recognized as antigens themselves. It has been shown that new tumor-specific antigens is an approximate time-consuming, costly and of limited effectiveness. Previous studies have shown that implantable microstructured 3-dimensional scaffolds can support the adhesion of macrophages and after implantation in vivo causes the necessary inflammatory reaction in the body accompanied by secretion of specific antibody, and development T- and B-cell memory. The proposed investigation is the use of the technology of silicon Scaffold in development of an immune response against cancer cells. To this end, we used recombinant Adenovirus to deliver the costimulatory molecule 41B and the chemokine CXCL10. After reaching the carcinoma 4T1 cells, the animals implanted with Adenovirus expressing CXCL10 showed a large regression compared to the control group. This method allows the natural selection of immunogenic epitopes for the development of specific cellular and humoral response against the tumor. The purpose of the research is to regulate the balance between/ host immunity and the development of individual specific response against the tumor. The application of such technology to humans will be of great importance opening novel areas of research and treatment.

P.B1.02.01
Tumor vaccination principles and Immunotherapy - Part 2
P.B1.02.02
Cessation of thymic activity impairs pTreg differentiation and enhances spontaneous and therapeutic tumor immune-surveillance
J. Almeida-Santos, M. Bergman, J. Cabral, I. Caramalho, J. Demengeot;
Instituto Gulbenkian de Ciência, Oeiras, Portugal.

It well established that depletion or inhibition of regulatory T cells (Treg) in mice and humans, favors immune rejection of solid tumors. While it has been proposed that both thymic- and peripherally-derived Treg (Treg and pTreg, respectively) can infiltrate the tumor environment, their relative contribution to tumor progression is still unclear. As recent thymic emigrants (TRE) have been shown to be the preferential precursors of pTreg in specific assays, we hypothesize that ongoing thymic activities play a role in tumor immune tolerance. To test this hypothesis, we assessed whether: i) the higher capacity of RTE to convert into pTreg is also true in a tumor context; ii) newly converted pTreg are required for tumor progression; and iii) thymectomy improves tumor-immunotherapies.

By performing adoptive transfer experiments into lymphopenic hosts, and testing several tumor models, we evidence that immature CD4 cells remain the preferential precursors of pTreg in a tumor context. Using genetically (DEREG) and surgically (Thymectomy) engineered mice to control Treg and RTE numbers in the periphery, we demonstrate that while depletion of Treg limits tumor growth, prevention of pTreg generation through elimination of RTE amplifies this effect. Moreover, we show that limiting thymic activities through thymectomy enhances the efficacy of anti-CTLA-4 immunotherapy.

In conclusion, our work suggests that natural or therapeutic thymic restriction may be beneficial in cancer treatment.

P.B1.02.03
Exploring novel anti-tumor roles of genome-damaging AID/APOBEC3 enzymes in breast cancer
M. Agharpour, M. Lorijani;
immunology and Infectious Diseases Program, Division of Biomedical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St John’s, Canada.

Introduction: AID/APOBEC3 cytidine deaminases are genome-editing enzymes that function to boost immunity. It is established that AID/APOBEC3s are an endogenous source of DNA damage leading to initiation and evolution of different cancer types, including breast cancer. We hypothesized that depending on expression levels, AID/APOBEC3s could also have anti-tumor functions.

Methods & Results: We established a first of its kind inducible expression system in which we control expression levels of AID, APOBEC3B and APOBEC3G in breast cancer cells. We verified enzymatic activity of each enzyme and inducible expression of RNA and protein was demonstrated by qRT-PCR, fluorescent microscopy, and flow cytometry. Using MTT, apoptosis and wound-healing migration assays, we observed both tumorigenic and anti-tumor effects depending on different expression levels of the enzymes. The observed effects of AID/APOBEC3s on tumor behavior were in accordance with gene expression levels of key factors involved in cancer cell death and migration as assessed by qRT-PCR.

Conclusion: AID/APOBEC3s play both pro- and anti-tumor roles depending on expression level. This finding represents a paradigm shift as AID/APOBEC3s could also have anti-tumor functions.

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POSTER PRESENTATIONS

P.B1.02.04 Human recombinant Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) secreted by Lactococcus lactis acts synergistically with cytostatics in elimination of human colon cancer cells in vitro.

K. Ciacho, J. Wieckiewicz, M. Siedlar, J. Baran;
Jagiellonian University Medical College, Krakow, Poland.

Introduction: One of the leading problems in the current treatment of colon cancer is resistance of the tumor cells to chemotherapy. TRAIL is a natural protein that effectively kills many types of tumor cells and potentially may act synergistically with some chemotherapeutics. However, the biological half-life of TRAIL in mammalian organism is very short, so it is very effective its therapeutic effectiveness. The aim of our study is to investigate, if non-pathogenic Lactococcus lactis bacteria can be used as a safe carrier of the TRAIL, enabling both, the control of TRAIL secretion over a period of time and elimination of tumor cells in vitro.

Methods: Recombinant plasmid harbouring hTRAIL-CDNA was constructed and transformed via electroporation into L. lactis NZ9000 cells. Synthesis and secretion of hTRAIL was determined in broth supernatants by PCR, ELISA and Western blot. Antitumor activity of hTRAIL in broth supernatant, used as a single agent and in combination with 5-Fluorouracil (5-FU), cetuximab (CPT-11), metformin (MetF), puromycin (Puro) against human colon cancer HCT116 cells was examined (in vitro) in MTS assay. Apoptosis of cancer cells was confirmed by Annexin V binding and flow cytometry analysis. Elimination of HCT116 cells in a co-culture with Lactococcus residues was assessed by MTS assay. Results: hTRAIL produced by L. lactis(hTRAIL+) effectively kills HCT116 cells and acts synergistically with cytostatics: 5-FU, CPT-11, MetF and Puro, enhancing elimination of colon cancer cells in vitro.

Conclusion: Lactococcus (hTRAIL+) bacteria can produce biologically active hTRAIL with potential application for colon cancer immunotherapy.

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P.B1.02.05 Bovine herpesvirus 4-based vector delivering the full length xCT DNA efficiently protects mice from mammary cancer metastases by targeting cancer stem cells Bovine herpesvirus 4-based vector delivering the full length xCT DNA efficiently protects mice from mammary cancer metastases by targeting cancer stem cells.

L. Conti1, G. Donadoni2, G. Tebaldi2, S. Landzard2, R. Ruvi2, E. Boli3, A. Ballatore3, V. Roli3, F. Macchi3, F. Cavollo3;
1MOLECULAR BIOTECHNOLOGY CENTER, TURIN, Italy, 2University of Parma, PARMA, Italy.

Despite marked advancements in its treatment, breast cancer is still the second leading cause of cancer death in women aged 20 to 59 years, due to relapses and distal metastases. Breast cancer stem cells (CSCs), are a cellular reservoir for recurrence, metastatic evolution and disease progression, making the development of novel therapeutics that target CSCs, and thereby inhibit metastasis, an urgent need. We have previously demonstrated that the cystine-glutamate antipporter xCT (SLC7A11), a protein that was shown to be overexpressed in mammary CSCs and that plays a key role in the maintenance of their redox balance, self-renewal and resistance to chemotherapy, is a potential target for mammalian breast cancer immunotherapy. We developed an anti-xCT viral vaccine that is based on the bovine herpesvirus 4 (BoHV-4) vector, which we have previously showed to be a safe vaccine that can transduce cells in vivo and confer immunogenicity to tumor antigens. We show that the vaccination of BALB/c mice with BoHV-4 expressing xCT (BoHV-4-mxCT), impaired lung metastases induced by syngeneic mammary CSCs both in preventive and therapeutic settings. Vaccination induced T lymphocyte activation and the production of anti-xCT antibodies that can mediate antibody-dependent cell cytotoxicity (ADCC), and directly impair CSC self-renewal and redox balance. Our findings pave the way for the potential future use of BoHV-4 vectors that target xCT in metastatic breast cancer treatment.

P.B1.02.06 High-dimensional profiling of immune subsets in mouse glioblastoma models reveals a tumor-induced ‘tolerogenic’ microenvironment.

VU University Medical Center, Amsterdam, Netherlands.

Tumor cells can manipulate their microenvironment to suppress tumor immunity, which affects patient survival. Modulation of inhibitory immune checkpoints through antibody therapy has shown promising results in several types of cancer, especially of those expressed by T cells. Glioblastoma is the most common, malignant form of primary brain cancer with a very dismal prognosis. As current therapies are insufficient, immune checkpoint inhibition could prove useful. Using advanced multiparameter flow cytometry and an unsupervised clustering algorithm, we performed a high-dimensional subset analysis and assessed co-localization of immune checkpoints and immune checkpoint ligands in two different mouse glioblastoma models. When comparing systemic effects with the brain tumor microenvironment and contralateral hemisphere of the same mouse, we observed a significant increase of CD4 T cells with a highly specialized phenotype, characterized by expression of TIGIT, PD-1, and, to a lesser extent, HVEM. PD-1 expression was also significantly increased on both OVA-antigen-specific CD8 T cells and non-OVA-antigen-specific CD8 T cells. Furthermore, analysis of the myeloid compartment showed massive infiltration of macrophages in the brain tumor microenvironment. Co-expression of immune checkpoint ligand and immune checkpoint molecules suggest a glioblastoma-induced ‘tolerogenic’ tumor microenvironment, within several different populations of infiltrating immune cells. Upregulation of certain immune checkpoints on lymphoid or myeloid or tumor cells, could serve as potential targets for combination therapy.

P.B1.02.07 Expansion and characterization of human tumor infiltrating lymphocytes (TILs) in colorectal cancer (CRC).

M. Españo1,2, R. Cabezón3,4, G. Floréz-Graví2, C. Esquiel5,6, E. Pineda2, A. Giné3, M. Juan1, J. Maurel7, D. Benitez-Ribas9;
10th Pathology Department, Hospital Clinic de Barcelona, Barcelona, Spain, 2Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, 3Institute of Immunogenetics, Biomedical Research, Madrid, Spain, 4Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, 51st Immunology, RIMLS, Radboudumc, Nijmegen, Netherlands, 6Medical Oncology Department, Hospital Clinic de Barcelona, Barcelona, Spain, 7Translational Genomics and Targeted Therapies in Solid Tumors Group, IDIBAPS, University of Barcelona, Barcelona, Spain, 8Department of Gastroenterology, CIBERehd, Hospital Clinic de Barcelona, Barcelona, Spain, 9Fundación Clínica, Barcelona, Spain.

Introduction CRC is one of the most frequent cancer worldwide and less than 40% patients remain free of progression after 12 months after diagnosis. Immunotherapeutic approaches to enhance anti-tumor T cell response have been developed, such as vaccination, immune-checkpoint inhibitors or adoptive cell therapy. Our aim was to expand and characterize CRC TILs as a preclinical work useful in tumour immunotherapy Methods

Eleven primary CRC tumour biopsies were obtained from 9 patients undergoing colonoscopy. Biopsies were obtained from 7 untreated patients and 2 patients before treatment and after first-line progressive disease. Biopsies were processed to obtain a cell suspension by mechanical and enzymatic dissociation. Cells obtained were plated for TIL growth and analyzed using BD-FACSDiva software.

Results TILs were obtained in 10/11 tumour samples. Generally, after 2-weeks of expansion a minimum amount of 4x10^6 TILs were expanded (mean 35,3x10^6). On average, 97.3% of TILs were CD3+, with a CD4/CD8 ratio of 9.6. Expression of PD1 and CTLA4 was 1.9% and 0.3% in CD4+ and 1% and 0.3% in CD8+, respectively. 47.8% CD4+ and 71% CD8+ expressed IFNγ, while 21.2% CD4+ and 37.5% CD8+ expressed IL17. No expression of IL10 was detected.

Conclusion This work shows our capability to obtain TILs and provides new data about the tumoral microenvironment in CRC that could be applied to design new therapies for these patients.

P.B1.02.08 Anti-migratory property of the dual delivery of SN38-Snail siRNA CMD-chitosan nanoparticles on prostate cancer cells

M. Farzí, A. Afkham1, S. Sadreddini1, S. Dolati1, N. Manafi Afkham1, M. Yousefi1;
1Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, TABRIZ, Iran, Islamic Republic of, 2Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, TABRIZ, Iran, Islamic Republic of.

Background: Prostate cancer is the leading cancer diagnosed in men in the US each year. Chitosan nanoparticles have become of great interest for nanomedicine, biomedical engineering and development of new therapeutic drug release systems specially in the USA. Snail and SN38 proteins have moved into the fast lane of development and cancer biology with the epithelial-mesenchymal transition (EMT). In the present study, we aimed to design chitosan/CMD nanoparticles for the efficient encapsulation of the anti-cancer drugs SN38 and Snail-specific siRNA. Methods: Physicochemical characteristics of the synthesized chitosan nanoparticles (140KD) were analyzed using Scanning Electron Microscopy (SEM). Serum & heparin stability and in vitro released assays were done. Anti-migratory property of the dual delivery of SN38-Snail siRNA CMD-chitosan nanoparticles was investigated through quantitative measurement of E-cadherin and Claudin-1 expression level in PC-3 human metastatic prostate cell line. The potential effects of the nanoparticles on migration capability of the prostate cancer cells was also assessed using wound healing assay. Results: Our findings evidently showed 3.12 (±0.62) and 3.02 (±0.28) fold increases in E-cadherin and Claudin-1 mRNA after 24h, respectively.
The upregulation of E-cadherin and Claudin-1 mRNA were continued to 5.6 (±0.91) and 4.42 (±0.61) fold after 48h, respectively. Moreover, co-delivery of SN38 and specific siRNA by chitosan nanoparticles resulted in significant anti-tumor activity. Conclusion: In conclusion, our results revealed that dual delivery of CNTNs encapsulating SN38 and Snail-specific siRNA may have a great impact on the treatment of prostate cancer.

P.B1.02.09
Polymer-mediated tumor immunotherapy by in situ activation of antigen presenting cells
J. Hahlbrock, D. Arnold-Schüll, J. Stickodorn, J. Braun, M. Brav, S. Grabbe, L. Nuhn, H. Schild; 1Institute for Immunology, Mainz, Germany, 2Max Planck Institute for Polymer Research, Mainz, Germany, 3Department of Dermatology, Mainz, Germany.

Immunotherapy has become a promising tool to treat cancer as shown by the use of checkpoint inhibitors. However, dependent on the tumor entity, there are still high recurrence rates and many patients suffer from immune related adverse events. Consequently, there is still high medical need for the development of specific tumor immunotherapies. We were able to design nanogel formulations which are degradable under acidic conditions and can be functionalized by covalent binding of the TLR7-agonist IMDQ and the tumor antigen OVA. Both, the nanoparticle itself and OVA are fluorescently labeled, which allowed biodistribution analyses. Intravenous application revealed an accumulation of the nanoparticles in the spleen with OVA-positive macrophages and elevated B cell numbers. Additionally, we found an uptake of functionalized nanoparticles in BMDCs as well as an enhanced BMDC maturation status. Interestingly, we observed a higher CD8+ T cell response after treatment with nanoparticles which were functionalized through covalent binding of IMDQ and OVA (NP(IMDQ+OVA)) compared to a high positive CD8+ T cell response after treatment with nanoparticles which contained IMDQ covalently bound but OVA added in a soluble form (NP(IMDQ)+OVA). In line with these results, we observed an increased IgG2a antibody production in sera of mice which were immunized with NP(IMDQ)+OVA and an increased CD8+ T cell response in splenocytes derived from mice treated with NP(IMDQ+OVA). Taken together, these results show promising effects of functionalized nanoparticles which involve CD4+ and CD8+ T cells as well as B cells. Next, therapeutic effects in mice carrying OVA-expressing tumors will be analyzed.

P.B1.02.10
Tumour escape in the microenvironment of penile carcinoma - PD-L1 related parameters predict lymph node metastases and survival
S. Ottenhof, N. Pocorni, R. Rajadalingham, H. Thygesen, J. de Jong, T. de Gruijt, S. Horelain, E. S. Jordanova; 1NK Cell Centre, Amsterdam, Netherlands, 2Dutch Cancer Center_AvL, Amsterdam, Netherlands, 3Center for Gynecologic Oncology Amsterdam, Amsterdam, Netherlands.

In the complex interplay between cancer and the hosts immune system, the tumour is threatened by the natural anti-tumour response of cytotoxic T-cells (CTL). However, CTLs are 1) inhibited by regulatory T-cells (Treg), 2) mislead by aberrant HLA expression by the tumour cells and 3) deactivated by Programmed Death Ligand 1 (PD-1/L) on tumour cells or on tumour infiltrating macrophages (TIFM). This study aims to gain insight in immunological factors and their prognostic value for lymph node metastases and disease specific survival (DSS) in penile cancer (n=213). HPV status, different levels of HLA expression and PD-L1 expression on tumour, stroma and TIM were known from previous studies. Sections were stained for macrophage-marker CD163, CTL-marker CD8, and Treg-marker FoxP. These parameters were included in multivariable regression models testing the prognostic value for lymph node involvement (LN+ or LN-) and DSS. Multivariable analyses showed three independently prognostic factors for both lymph node status and DSS: 1) PD-L1+ TIM (odd ratio 2.41, p=0.003), 2) PD-L1+ tumor cell margin expression (odd ratio 2.86, p=0.002), and a high intra-tumoral CTL/Treg ratio (odd ratio 1.23, p=0.044). This means that PD-L1+ TIM and PD-L1 margin expression are independently predictive in absence of HPV. A PD-L1 expression pattern predominantly at the tumour-stroma margin predicts good prognosis, while the negative predictive value of PD-L1+ TIM appear to be compensated by a high CTL/Treg ratio. These results strengthen the rational for anti-PD-1/PD-L1 immunotherapy in penile carcinoma.

P.B1.02.11
Drug-induced hyperploidy stimulates an antitumor NK cell response mediated by NKG2D and DNAM-1 receptors

Introduction: Formation of polyploid or aneuploid cells is a pathological hallmark of malignant tumors. In addition to cell cycle checkpoints, cancer cell DNA ploidy is subjected to extrinsic controls operated by activation of T-cell mediated immune responses. Whether the innate immune system, and specifically natural killer (NK) cells, have a role in this process has not been deciphered yet.

Materials and Methods: Tumor cell lines were exposed to hyperploidy-inducing agents and surface expression of NK cell ligands was analyzed by flow cytometry. NK cell cytotoxic activity and cytokine production was evaluated after co-culture with treated tumor cells. Pharmacological and genetic approaches were used to study the intracellular mechanism involved in the upregulation of NK cell ligands in hyperploid cancer cells.

Results: Herein, we report that drug-induced polyploidy in cancer cells activates antitumor responses mediated by NK cells. Hyperploidy-inducing agents strongly upregulate the surface expression of NKG2D and DNAM-1 ligands in tumor cells. Further, drug-induced hyperploidy modulates the repertoire of activating receptors and the cytokine profile of NK cells. Moreover, susceptible NK cell-mediated hyperplasia is stress-related signaling pathways, DNA damage and endoplasmic reticulum stress responses, were involved in the stimulation of MICA, a key NKG2D ligand in hyperploid cells.

Conclusion: Overall, our findings indicate that, besides the cytotoxic effect on tumor cells, the therapeutic activity of anti-mitotic drugs can also be mediated by the induction of a coordinated antitumor immune response involving NK cells.

P.B1.02.12
TARGETING C70 FOR THE TREATMENT OF B CELL LYMPHOMA

C70 is a member of the TNF family that is typically only transiently expressed on several types of immune cells in settings of immune activation. It ligates its receptor CD70, while knock-out lines will serve as negative controls. Cell proliferation and survival of treated and untreated CD70 knockout cells in combination with various anti-cancer therapies will be measured. In our experiments, we will test the function of C70-positive and -negative cell lines in mice by monitoring lymphoma growth, drug sensitivity and their impact on the tumor microenvironment. Our preliminary analysis suggests that stimulation of C70 on cancer cells may promote the immune suppressive cytokine IL-10.

P.B1.02.13
Repurposing antiviral T cells to fight tumors

Overcoming the immunosuppressive tumor microenvironment remains a major impediment to successful cancer immunotherapy. Virus-specific memory T cells are positioned throughout the entire body to sense reinfec tion and rerudescence. When that same virus is reencountered, these T cells sound an alarm that induces a local immunostimulatory environment that activates and recruits many arms of the immune system. As memory T cells are present in abundance in nearly every tissue and can be triggered by cognate virus-specific peptides they recognize, they are well-positioned to respond to several causes of immunity. However, there is still much to be learned about the function of CD70 activation in cancers and its downstream signaling pathway(s) is unknown.

The aim of this project is to study the role of CD70 in B cell lymphomas and multiple myeloma and to investigate how cancers benefit from the expression of this protein. So far, we have generated a couple of C70-deficient cell lines using CRISPR/Cas9 technology. Genome-wide expression arrays will be performed to identify downstream targets of CD70, while knock-out lines will serve as negative controls. Cell proliferation and survival of treated and untreated C70 knockout cells in combination with various anti-cancer therapies will be assessed by flow cytometry. Finally, we will test the function of C70-positive and -negative cell lines in mice by monitoring lymphoma growth, drug sensitivity and their impact on the tumor microenvironment. Our preliminary analysis suggests that stimulation of C70 on cancer cells may promote the immune suppressive cytokine IL-10.
P.B1.02.14
The combination of magnetic nanoparticles and magnetic fields induces T cell retention both in vitro and in vivo
L. Sanz-Ortega1, J. M. Rosaj1, A. Marcas1, Y. Portilla1, J. V. Steijn2, D. F. Barber3
1Department of Immunology and Oncology, Centro Nacional de Biotecnología, Madrid, Spain, 2Theodor Kocher Institute, Bern, Switzerland

Introduction: A main limitation in cell-based therapies is the dispersion of the administered cells resulting in a small proportion reaching the site of interest. Manipulating cells to target a region could be a strategy to solve this problem. T cells loaded with magnetic nanoparticles (MNPs) could be used for this purpose as long as their functions are not impaired. Here, we evaluate whether MNPs could serve to magnetically guide T cells. Methods: Jurkat and murine T cells were used to assess several aspects of T cells after MNP treatment. In vitro experiments were performed with T cells together with DCs; these experiments were designed to explore the failure to in vivo manipulation of MNPs (TILs). The in vitro and in vivo manipulation of MNPs with an external magnetic field (EMF) was also analyzed. Results: MNPs remain mainly in the cell membrane of T cells. MNPs did not alter cell surface markers expression but slightly reduce the chemotactic response, which can be enhanced using EMFs. EMFs can also enhance the in vitro retention of MNP-loaded cells in flow conditions. Moreover, we observed an in vivo accumulation of T cells in the lymph nodes (LNs) promoted by MNP loading and enhanced by localized EMFs. Finally, MNPs and EMF can reduce the speed of naïve T cells in the LNs. Conclusions: This work shows the use of MNPs and EMFs to guide and retain T lymphocytes to certain regions without affecting crucial biological aspects. These studies reveal an interesting approach to promote cell retention that could be implemented in cell-based therapies to improve their efficacy.

P.B1.02.16
Reinforcing dendritic cells for cancer immunotherapy: diverse aims to target antigens to human skin
1Medical University of Innsbruck, Innsbruck, Austria, 2Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, 3Newcastle University, Newcastle, United Kingdom, 4University of Zurich, Zurich, Switzerland

Dendritic cells (DC) are essential for the induction of primary immune responses, and hence preferred targets for immunotherapy of cancer. Skin DC express C-type lectin receptors such as Langerin or DEC-205 for antigen capture. Langerin is expressed mainly on Langerhans cells (LC), whereas DEC-205 is expressed by dermal DC and LC. We aim to load skin resident DC with antibody-antigen fusion proteins directed against these C-type lectin receptors or with antigens encapsulated in liposomes coated with a Langerin ligand. Langerin monoclonal antibody (mAb) injected intradermally into human skin explants was detected exclusively in LC, whereas DEC-205 mAb targeted both dermal DC and LC. A model antigen (EBNA1) fused to DEC-205 mAb elicited EBNA1-specific T cell responses. Liposomes coated with a Langerin ligand showed exclusive binding to LC in cell suspensions obtained from healthy human skin. These liposomes were rapidly incorporated into LC as visualized by confocal microscopy. Furthermore, to test our vaccination approaches in an in vitro model, we generated monocyte-derived Langerhans-like cells, which displayed between 50-80% of Langerin expression on the surface and also showed upregulation of CD83 and HLA-DR upon stimulation with a maturation cocktail. In summary, our study will provide a deeper insight into DC-targeted cancer vaccines, their uptake, intracellular trafficking and antigen processing in skin DC. Furthermore, liposomes provide a flexible platform that will allow us to encapsulate antigens to investigate their potential for targeted delivery. Ultimately, this DC-based immunotherapy can be used to increase response rates when used in combination with immune checkpoint inhibitors.

P.B1.02.17
Phenotypic patterns of tumor-infiltrating T and NK cells reflect tumor grading in renal carcinoma
Z. Strizova1, R. Taborska1, D. Stakhveev2, K. Havlova2, V. Vesely2, J. Bartkunova2, D. Smrz2
1Department of Immunology, 2nd Faculty of Medicine, Charles University in Prague and Motol University, Prague, Czech Republic, 2Department of Urology, 2nd Faculty of Medicine, Charles University in Prague and Motol University, Prague, Czech Republic

Infiltrating immune cells are mechanistically involved in the anti-tumour effect of this novel treatment. Re-challenge experiments also showed that immune memory was induced. Flow cytometry combination treatment showed significantly increased survival, with 60-80% of the mice being completely tumour free. Depletion studies revealed both CD8+ T cells and NK marker 4-1BB upon exposure to tumor digests and no or lower reactivity to healthy kidney tissue from the same patient. The tumor-specific upregulation of other activation markers (CD40L, CD107a, PD1) was only found in a subset of patients.

P.B1.02.18
Expanded tumor infiltrating lymphocytes upregulate 4-1BB in response to renal cell carcinoma
S. D. van Asten1,2, R. de Groot1,3, M. M. van Laenen1,4, A. Been1, De Jong1, D. Amens1, R. M. Spaepen1, C. M. Wolters1
1Dept of Oncoanatomy, Sanquin Research, Amsterdam, Netherlands, 2Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands, 3Dept. of Hematopoiesis, Sanquin Research, Amsterdam, Netherlands, 4The Netherlands Cancer Institute, Amsterdam, Netherlands

The activation of autologous tumor infiltrating lymphocytes (TILs) is a promising approach in the treatment of multiple cancers. However, its efficacy in treatment of renal cell carcinoma (RCC) is low. Low efficacy is caused by a failure to obtain highly potent tumor reactive lymphocytes after expansion of tumor-infiltrating lymphocytes (TILs). Therefore selective expansion of TIL populations with a potent cytotoxic and migratory activity is needed. In our study we analyzed the localization of cytotoxic/migratory NK, NKT and T cells infiltrating the tumor, peritumoral and adjacent healthy renal tissue. METHODS: 14 patients who underwent radical nephrectomy were included in the study. 42 tissue samples were obtained from the kidney, the tumor and peritumoral tissue, sliced and enzymatically dissociated into single cell suspensions. These cells were then analyzed for the expression of established markers (CD40L, CD107a, PD1) by flow cytometry and the findings correlated with clinical and histopathological data. RESULTS: A tendency to a higher tumor infiltration with PECA-1+1-Fasl + NKT and NK cells was observed in low grade tumors (grade 2) rather than in poorly differentiated high grade tumors (grade 3). In NK cells, this trend was significant (P=0.003). Moreover, PECA-1+1-Fasl + NK cells were most frequent in peritumoral tissue. CONCLUSION: The frequency of cytotoxic/migratory NK, NKT and T cells within the tumor and the surrounding milieu is affected by tumor grading. These results may provide important information for development of TIL expansion protocols for ACI of RCC.

P.B1.02.19
Combined immune stimulation with IL-15 and CD40 results in profound anti-tumour effects in pancreatic cancer
J. R. M. Van Audenaerde1, B. Von Scheidt2, A. Unsworth2, E. Marcelli3, O. Oliver2, J. De Waele1, G. Roesen1, C. Y. Slaney1, A. R. K. Darcy2, M. Peeters4, M. H. Kershaw1, E. L. Smith1
1Center For Oncological Research, University of Antwerp, Wilrijk, Belgium, 2Cancer Immunotherapy and Immune Innovation Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia, 3Dep. of Hepatobiliary, Endocrine and Transplantation Surgery, Antwerp University Hospital, Antwerp, Belgium, 4Dept of Oncology and Multidisciplinary Oncological Centre Antwerp, Antwerp University Hospital, Antwerp, Belgium, 5Center for Cell Therapy and Regenerative Medicine, Antwerp University Hospital, Antwerp, Belgium

Background: Pancreatic cancer (PC) is the 3rd deadliest cancer worldwide with the lowest 5-year survival of all cancers. Therapeutic improvements have barely been made over the last decade. Within the tumour microenvironment, targeting the stromal shield is needed to overcome treatment resistance. CD40 stimulation has already demonstrated moderate anti-tumour responses in PC, including some anti-stroma effects. We have shown that interleukin (IL)-15 stimulated NK cells are capable of tackling both tumour as well as the surrounding desmoplastic stroma. Therefore, we explored a novel combination immunotherapy consisting of an agonistic anti-CD40 monoclonal antibody and IL-15 in two mouse models of PC.

Methods: C57BL/6 mice bearing subcutaneous Panc02 or KPC tumours were treated over a two-week period with IL-15 and/or anti-CD40. Tumour kinetics and survival were monitored. Experiments depleting different immune cell populations were performed. Re-challenge experiments were executed to check immune memory induction. Tumour infiltrating lymphocytes are being characterised using flow cytometry and flow immunohistochemistry. Re-challenge combination treatment of IL-15 and anti-CD40 caused a distinct reduction of tumour growth rates in comparison with single agent treatments. Mice receiving the combination treatment showed significantly increased survival, with 60-80% of the mice being completely tumour free. Depletion studies revealed both CD8+ T cells and NK cells are mechanistically involved in the anti-tumour effect of this novel combination treatment. Re-challenge experiments also showed that immune memory was induced. Flow cytometry experiments and immunohistochemistry experiments are being performed to provide more details on the phenotype of the tumour infiltrating lymphocytes and their spatial distribution within the tumour.

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Poster abstracts

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

218
POSTER PRESENTATIONS

P.B1.02.20 Induction of immunogenic cell death by innovative antitumoral platinum (II) compounds

M. Wanta1, N. Chekkt1, M. Bouché2, G. Dahn1, B. Frisch3, S. Bellerin-Laponnaz2, S. Fourne1;
1Faculty of Pharmacy, Iilkærk Cédez, France, 2IPCMS, Strasbourg Cédez, 2, France.

Some cancer treatments like chemotherapeutic agents (anthracyclines, platinum derivatives, ...) are able to activate the antitumor immune response by inducing a particular cell death: the immunogenic cell death (ICD). This process is characterized by the exposition of the endoplasmic reticulum chaperone calreticulin (CRT) at the cell surface as well as the release of ATP and non-histone chromatin-binding protein high mobility group box 1 (HMGB1) which serve as immuno-stimulatory damage-associated molecular patterns (DAMPs) and increase the antitumor immune response. We focused on N-heterocyclic carbene platinum complexes associated with polyethyleneimine, a transfection agent, to create multivalent cationic platinum compounds (NHC-PEI(II)-PEI) that induce apoptosis in vitro and in vivo in xenograft immunodeficient mouse model1. To evaluate the potential implication of the immune response on the NHC-PEI(II)-PEI in vivo effect, immunocompetent mice bearing tumors were treated with platinum particles and the results revealed an antitumor effect of our conjugates, in the same range than the clinical used platinum drug oxaliplatin, but with less side effects. We evaluated if NHC-PEI(II)-PEI were able to induce ICD. First results showed expression of CRT upon NHC-PEI(II)-PEI treatment. We are then evaluating if their association with immune danger signals could enhance this effect. Altogether our results reveal the possibility of creating Pt(II) derivatives that can be used as chemotherapeutic agents by killing tumor cells and as immunotherapeutic agents by triggering the antitumor immune response.

1 Chekkt et al, Bioconjugate Chem 2016, 27, 1942-1948

P.B1.03.01 Molecular mechanism for M1 bias of ABCG1-deficient macrophages

M. Altunay1, S. Gunalp2, D. Unvar Purcu1, R. Ozbilgin1, Z. Aydi1, F. Hap1, Z. Tasan2, G. Wingender1, D. Sag1;
1Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey, 2Department of Molecular Medicine, Health Sciences Institute, Dokuz Eylul University, Izmir, Turkey.

Macrophages that are major players of tumor immunity, are divided into two subgroups as M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages. In general, M1 macrophages are potent tumor-fighting cells, whereas M2 macrophages display protumoral functions. ATP-binding Cassette Transporter G1 (ABCG1) promotes cholesterol efflux from cells and regulates intracellular cholesterol homeostasis. We have recently shown that in the absence of ABCG1, macrophages shift from a tumor-promoting M2 phenotype to a tumor-fighting M1 phenotype within the tumor and suppress bladder cancer growth in vivo. The molecular mechanism through which ABCG1-deficiency shifts macrophage polarization to an M1 phenotype is not known. AMP-activated protein kinase is a master regulator of energy metabolism. In macrophages, AMPK also regulates M1/M2 polarization and activation of AMPK promotes macrophage polarization to an M2 phenotype. Herein, we show that ABCG1-/- bone marrow-derived macrophages display reduced levels of AMPK activation at baseline and also after stimulation with LPS/IFNγ (M1 stimulus) or IL4 (M2 stimulus). Moreover, while ABCG1-/- macrophages stimulated with LPS/IFNγ have increased TNFα production compared to WT macrophages, after treatment with the AMPK activator AICAR, the TNFα production of ABCG1-/- and WT macrophages were comparable. Our data suggest that the M1 bias of ABCG1-/- macrophages is mediated through AMPK signaling pathway. These findings not only deepen our mechanistic understanding of the M1/M2 switch in macrophages, but have the potential to open new up immunotherapeutic approaches for the treatment of cancer.

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P.B1.03.02 Adoptive cell transfer following personalized cancer vaccination elicits newly detectable neo-antigen-specific T-cell responses in ovarian cancer patients

V. Bianchi1, S. Bobisse1, B. J. Stevenson1, L. Kandalaf1, A. Harari1, G. Coukos1;
1 Ludwig Institute for Cancer Research, Lausanne, Switzerland, 2Swiss Institute of Bioinformatics, Lausanne, Switzerland.

Private tumor neo-antigens derived from non-synonymous somatic point mutations can be immunogenic and are becoming highly attractive targets for tailored mutanome-based immunotherapies. A pilot study conducted by Kandalaf et al. and colleagues, has shown that a personalized vaccination strategy in advanced ovarian cancer patients is feasible and safe, and induces a broad antitumor response including T-cell reactivities against private mutated neo-epitopes (Kandalaf et al., 2013; Tanji et al. 2018). Patients who developed antitumor T-cell responses following vaccination but failed to achieve a complete remission (n=12), were enrolled in a second part of the trial involving lympho-depletion and adoptive cell transfer (ACT) of autologous vaccine primed ex vivo co-stimulated T-cells (Kandalaf et al., 2013). To investigate whether ACT provided a boost to the cancer vaccine treatment, we performed a longitudinal screening of neo-epitope specific T-cells in the peripheral blood of vaccinated patients who received T-cell infusion. Of interest several (n=6) CD8+ T-cell responses to private nonsynonymous mutations (neopeptides) were newly detected only upon ACT in the blood of 4 out of 11 patients. Furthermore, increased progression-free survival was associated with the de novo detection of neopeptide-specific T-cell responses after ACT. We are now in the process of further dissecting the frequency of neoantigen-specific T-cell populations in the T-cell infusion products of patients undergoing ACT. Tracking the origin, expansion and persistence of neo-antigen specific T-cell clonotypes upon infusion, will help elucidate whether ACT of ex vivo co-stimulated T-cells following personalized vaccination is an effective way to further mobilize T-cell reactivities against the patient's mutanome.

P.B1.03.03 Interferons synergize with either TLRs or CD40-induced signaling to efficiently render macrophages tumoricidal in vitro

P. F. Christopoulos1, A. Lunde1, E. Müller2, T. A. Theodossiou1, B. J. Stevenson1, E. Müller2;
1Tumor Immunology Lab, Department of Pathology, Rikshospitalet, Oslo University Hospital, Oslo, Norway, 2Department of Radiation Biology, Institute for Cancer Research, Radium Hospital, Oslo University Hospital, Oslo, Norway.

Introduction: TAMs represent a main component of the tumor-infiltrating immune cells and therefore re/polarization into their anti-tumor M1 phenotype has raised great interest in cancer immunotherapy. IFNα/and or LPS have been described as the typical inducers of the classical M1 activation, however less is known about the effect of other molecules or TLR ligands on macrophages activation.

Methods: LPS, Pam3CSK4, IFNγ, IFNβ and sCD40L were used alone or in combinations to activate mouse BMDM. Production inhibition and/or apoptosis of LLC and MOPC315 cells were investigated using co-culture in vitro assays. Production of NO and cytokines, as well as, other phenotypic properties of polarized BMDM were also analyzed.

Results: We found that IFNγ induced growth inhibition and apoptosis of cancer cells only when it was used in combination with TLR agonists or sCD40L. Similarly, IFNβ also synergized with TLR ligands for induction of cancer cell growth inhibition. In addition, combinational treatments synergistically upregulated NO, as well as TNFα and IL-12 production in BMDM, whereas IL-10 secretion was suppressed. Furthermore, activated BMDM upregulated CD38, CD40, CD80/86, MHCI/II and PD-L1 in different expression patterns depending on the applied stimuli. The mitochondrial respiration was suppressed upon activation and to a greatest extent following combinational treatments. Finally, upregulation of macrophages was not greatly affected by activation.

Conclusions: Herein we have shown that TLR agonists, sCD40L and/or interferons promote distinct functional and phenotypic properties in so-called M1 macrophages. We demonstrate that activation of more than one signaling pathway is required to efficiently induce macrophage tumoricidal in vitro.

P.B1.03.04 Astragaloside II Exerted Anti-Tumor Immunological Effect through Regulating CD45

w. Chunping;
Yunnan University of Traditional Chinese Medicine, Kunming, China.

Object: This paper was designed to assess the anti-tumor immunological effect and reveal the molecular mechanism on Astragaloside II exhibit the anti-tumor immunological effect via regulating CD45.

Method: The H22 tumor-bearing mice was established. Mice were divided into three groups including, Model group, Astragaloside II group and Astragaloside II+Anti-CD45 Ab group. After 10 days of intragastric administration, the tumor tissue were isolated to assess the anti-tumor immunological effect of Astragaloside II. The lymphocyte cells proliferation from H22 tumor-bearing mice was detected by MTT method. The mRNA expression of Th1 cytokine, Th2 cytokine and transcript factor T-bet were examined by q-PCR analysis. Surface markers (Th1 and Th1 intracellular cytokines) (IFN-γ) were detected by flow cytometry.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 219
Abstracts of the 5th European Congress of Oncology - ECO 2018 - Amsterdam, The Netherlands

**Result:** The tumor weight, tumor diameter and tumor volume in Astragaloside II group are more than small model group (p < 0.05). Astragaloside II treatment significantly enhanced the immune-stimulatory activity of CD4+ CD69+ effector T cells, up-regulated the transcriptional expression in Th1 and Th2, including IFN-γ, IL-2, IL-4 and T-bet. Meanwhile, Astragaloside II treatment also markedly increased IFN-γ expression in CD4+ T cells and down-regulated T regulatory cell expression. Furthermore, anti-mouse CD45 Ab treatment intensely blocked the anti-tumor immunological effect and lymphocyte proliferation activity which induced by Astragaloside. Compared with Astragaloside II group, IL-2, IFN-γ, IL-4 and T-bet mRNA expression is decreased in Astragaloside II+Anti-CD45 Ab group. Anti-mouse CD45 Ab treatment intensively down-regulated IFN-γ expression in CD4+ T cells without significantly affecting Treg cells and CD4+ CD25+ effector T cells.

**Conclusion:** We hypothesis that activating CD45 expression in tumor tissue may be involved in anti-tumor immunological effect of Astragaloside II.

**P.B1.03.05**

Targeted human skin DC using melanoma specific multivalent glyco-nanomers to enhance anti-tumor immune responses


Human skin dendritic cell (DC) subsets are actively explored for use in anti-cancer vaccination strategies because of their easy accessibility. Targeting epidermal Langerhans cells (LCs) or dermal CD1a+, CD14+, and CD141+ DCs individually has shown enhanced (cross-) presentation of tumor associated antigens (TAA), inducing tumor specific CD4+ and CD8+ T cells, yet not enough for effective cancer regression in humans. We hypothesize, that simultaneous targeting of multiple DC subsets might give superior responses in vivo. We designed multivalent glyco-nanomers which showed increased CD8 T cell responses compared to single peptides in vivo, showing the potential for use of multivalent CLR targeting moieties to induce anti-tumor T cell responses. To verify targeting capabilities of these nanomers specifically to human skin DCs a human skin explant model was used. Multivalent glyco-nanomers containing gp100 MHC-I and II epitopes were coupled to the common DC-SIGN/Langerin ligand Le, thereby targeting both LCs and dermal dDCs (dDCs). We show increased uptake by LCs, CD14+ and CD141+ DCs in vitro, which, surprisingly, was only enhanced in vitro CD8+ T cell activation. To ensure proper T cell activation we combined the multivalent nanomers with the PRR agonists MPLA (TLR4) and NOD2 (DDO2). We showed an altered skin environment milieu when MPLA and NODP-nanomers are injected in situ in human skin and enhanced in vivo CD8 T cell responses in B6 mice. In conclusion, we designed tumor specific multivalent glyco-nanomers which can target both CLR and PRR on multiple human skin DC to induce anti-tumor immune responses.

**P.B1.03.06**

Reduced DNA damage and impaired function of cytokine-secreting and cytotoxic NK cells in tumor draining lymph nodes in non-small cell lung cancer

E. Cetin-Akta, A. Turmaz, F. Eezen, A. Engin, M. Agktor, G. Onur; 1Istanbul University, 2Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey; 3Istanbul University, Cerrahpaşa Medical School, Department of Thoracic Surgery, Istanbul, Turkey; 4Istanbul Medeniyet University School of Medicine, Department of Ophthalmology, Istanbul, Turkey; 5Istanbul University, Cerrahpaşa Medical School, Istanbul, Turkey.

Natural killer (NK) cells have important functions in antitumoral immunity and have been shown to have impaired functions in cancer patients. The aim of this study was to evaluate predefined immune responses in tumor draining lymph nodes of non-small cell lung cancer (NSCLC). Five NSCLC lung cancer patients undergoing preoperative mediastinal staging by video-assisted mediastinoscopic lymphadenectomy were included in this study. Peripheral blood mononuclear cells were isolated from tumor draining lymph nodes and peripheral blood. Activator and inhibitory receptors, immune checkpoint molecule expressions were analyzed in NK cell subsets. Cytotoxicity and cytokine secretion of NK cells were also analyzed. The rate of cytotoxic CD56dimCD161+ NK cells (p=0.008) and CD16+CD56int NK cells (p=0.03) were significantly diminished in lymph nodes, while there was no difference for cytokine-secreting NK cells (p=0.22). II-10 secreting NK cells were significantly increased (p=0.02), while IFN-γ (p=0.03) and TNF-α (p=0.03) secreting CD16 CD56+ effector cells were significantly diminished in lymph nodes, representing a regulatory phenotype. Percentages of CD16–/CD56+ (p=0.047) and granzyme expression of CD16–/CD56+ in unstimulated and K562 stimulated conditions (p=0.016 and p=0.008) were significantly lower in lymph nodes, while there was no statistically significant difference for CD107a degranulation (p=0.20) and p=0.10 in stimulated conditions. In addition, we found that CD56dim NK cells are significantly lower in lymph nodes (p=0.047). This study demonstrated decreased lymph node infiltration of NK cells, which might be associated with antigen-specific antibody responses. Exosome-injected mice demonstrated antigen-specific memory after 4 months. To be noted, mice receiving double allogeneic exosomes have so far only showed moderate T cell responses, suggesting a need for optimization of exosome-induced immunity in humans. We previously demonstrated that induction of antigen-specific CD8+ T cells and anti-tumor responses to whole antigen were independent of major histocompatibility complex (MHC) class I on exosomes. Here, we further investigated humoral and cellular immunity induced by syngeneic and allogeneic exosomes. Both exosomes can enhance antigen-specific CD8+ T cells, follicular helper T cell (Thf) and antigen-specific antibody responses. Exosome-injected mice demonstrated antigen-specific memory after 4 months. To be noted, mice receiving double allogeneic exosome injections showed highest antibody avidity. Reduced B16MOMA melanoma tumor growth was shown in all exosome-injected groups. Our findings support the application of these exosomes for therapeutic use in clinical immunotherapy studies. This work was supported by grants from the Swedish Research Council Medicine, The Swedish Cancer Foundation, The Cancer Research Foundations of Radiumhemmet, The Swedish Heart-Lung Foundation, Centre for Allergy Research Karolinska Institutet, and the Karolinska Institutet.

**P.B1.03.07**

Exosome based immunotherapy to induce antigen-specific humoral and cellular immunity and mediate long-term memory in vivo

X. Hu; G. Guculuer, R. Veerman, P. Larsen, S. Gabrielsen; Karolinska Institutet, Sollentuna, Sweden.

Exosomes are candidates for cancer immunotherapy due to their capacity to stimulate tumor-specific activity in vivo. However, clinical trials using peptide-loaded autologous exosomes have so far only shown moderate T cell responses, suggesting a need for optimization of exosome-induced immunity in humans. We previously demonstrated that induction of antigen-specific CD8+ T cells and anti-tumor responses to whole antigen were independent of major histocompatibility complex (MHC) class I on exosomes. Here, we further investigated humoral and cellular immunity induced by syngeneic and allogeneic exosomes. Both exosomes can enhance antigen-specific CD8+ T cells, follicular helper T cell (Thf) and antigen-specific antibody responses. Exosome-injected mice demonstrated antigen-specific memory after 4 months. To be noted, mice receiving double allogeneic exosome injections showed highest antibody avidity. Reduced B16MOMA melanoma tumor growth was shown in all exosome-injected groups. Our findings support the application of these exosomes for therapeutic use in clinical immunotherapy studies. This work was supported by grants from the Swedish Research Council Medicine, The Swedish Cancer Foundation, The Cancer Research Foundations of Radiumhemmet, The Swedish Heart-Lung Foundation, Centre for Allergy Research Karolinska Institutet, and the Karolinska Institutet.

**P.B1.03.08**

Ectopically expressed membrane-bound form of IL-9 exerts immune-stimulatory effect on CT26 colon carcinoma cells

V. Do Thi, Y. Kim; Department of Biochemistry, College of Natural Sciences, Chungnam National University, Daejeon, Korea, Republic of.

IL-9 is a known T cell growth factor with pleiotropic immunological functions, especially in parasite infection and colitis. However, its role in tumor growth is controversial. In this study, we generated tumor clones expressing the membrane-bound form of IL-9 (MB-IL-9) and investigated their influences on immune system. MB-IL-9 tumor clones showed reduced tumorigenesis but shortened survival accompanied with significantly decreased body weight loss in mice. MB-IL-9 expression on tumor cells had no effect on cell proliferation or major histocompatibility complex class I expression in vitro. MB-IL-9 tumor clones were effective in amplifying CD4+ and CD8+ T cells and increasing cytotoxic activity against CT26 cells in vivo. We also observed a prominent reduction in body weights and survival period of mice injected intraperitoneally with MB-IL-9 clones compared with control groups. Ratios of IL-17 to interferon (IFN)-γ in serum level and tumor mass were higher in mice implanted with MB-IL-9 tumor clones than those observed in mice implanted with control cells. These results indicate that the ectopic expression of the MB-IL-9 on tumor cells exerts an immune-stimulatory effect with toxicity. To exploit its benefits as a tumor vaccine, a strategy to control toxicity of MB-IL-9 tumor clones should be developed.

**P.B1.03.10**

Immunomonitoring of triple negative breast cancer patients undergoing neoadjuvant therapy (GBG98, Geparnuovo trial)

C. Massar1, A. Muller2, A. Schneeweiss1, C. Hanusch1, J. Huober1, M. Untch3, T. Kapp4, P. Fasching5, F. Marmé1, N. Burchardi1, C. Denkert1, S. Loibl2, B. Seigler1; 1Martin Luther University, Halle (Saale), Germany, 2National Centrum für Tumorerkrankungen, Heidelberg, Germany, 3Robert-Koch-Institut, Berlin, Germany, 4University of Ulm, Ulm, Germany, 5HÉLIO Klinikum Berlin Buch, Berlin, Germany, 6University of Frankfurt, Frankfurt, Germany, 7University Hospital Erlangen, Erlangen, Germany, 8GBG German Breast Group, Neu-Ignensburg, Germany, 9Charité University Hospital, Berlin, Germany.

The Geparnuovo trial is a randomized, double-blind, multicenter Phase II trial of neoadjuvant therapy in patients with early-stage triple negative breast cancer (TNBC) investigating the role of dulvaluam, an anti-PD-L1 inhibitor in addition to standard chemotherapy with nab-Paclitaxel followed by Epirubicin plus Cyclophosphamide. In flow cytometry, we determined possible predictive and/or prognostic biomarkers, blood samples were taken before and during the different treatment phases and evaluated by multicolor flow cytometry. Evaluation of the absolute cell count in the whole blood highlighted a mixed behavior of the total leukocytes, whereas there was a statistically significant reduction in the lymphocytes, particularly during the last phase of the treatment. Further dissection into the different immune populations highlighted an almost complete loss of B cells that in some patients was also accompanied by a reduction of NK cells, mostly regarding the CD161 subset. However, the loss of CD161 cells in B6 mice has been less pronounced resulting in an overall enhancement of their percentages within the total lymphocytes. The different populations have also been evaluated for the expression of activation and exhaustion markers, whose behavior will be more deeply evaluated when the clinical outcome and the treatment received by the patients will be made available. We expect that with such a comprehensive biomarkers for the treatment of TNBC patients will be identified thus leading to better patient selection for tumor chemo/immuno combination therapy. The implementation and translational research project was funded by AZ and Celgene, Germany, respectively.
POSTER PRESENTATIONS

P.B1.03.11
Flagellin increases death receptor-mediated cell death in a RIP1-dependent manner

1Department of Immunology, University of Debrecen, Debrecen, Hungary; 2Immunology Department, University Eötvös Lorand, Budapest, Hungary; 3Institut de Biologie Valrose, CNRS UMR 7277, INSERM UMR, Université de Nice, France; 4Department of Bioengineering, Sapientia Hungarian University of Transylvania, Cluj-Napoca, Romania.

Efficient adjuvants have the potential to trigger both innate and adaptive immune responses simultaneously. Flagellin is a unique pathogen-derived protein, which is recognized by pattern recognition receptors (PRRs) as well as by B-cell and T-cell receptors thus providing an important link between innate and adaptive immunity.

Herein we sought to investigate the potential modulatory effects of flagellin exerted on various cell death processes known to play detrimental roles in regulating the final outcome of various types of immune responses. We proved that the pre-treatment of Jurkat T-cells with flagellin is able to increase the degree of cell death provoked by FAS, TRAIL or TNF-α and concomitantly increases the cytotoxic potential of phytohaemagglutinin activated T-lymphocytes. In contrast to these flagellin-modified effects exerted on the death receptor-induced signalling events, the mitochondrial apoptotic pathway remained unaffected. Furthermore, the cell culture supernatant of wild type Salmonella enteritidis bacteria, but not their flagellin deficient variant were able to enhance the Fas-induced cell death. To define the molecular mechanisms mediated by flagellin we were able to detect the upregulation of RIP1-dependent signalling events.

These findings demonstrate that the cooperative action of pattern recognition and the different death receptors are able to initiate the cell death process towards the mobilization of RIP-dependent cell death modalities. This finding highlights the capability of flagellin to act as a potential adjuvant relevant for tumor immunotherapy.

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P.B1.03.12
Cytokine nitration boosts myeloid suppressor cell commitment and functions in tumors

1Department of Biomedical Sciences, University of Padova, Padova, Italy, 2Venetian Institute of Molecular Medicine VIIIM, Padova, Italy, 3ShanghaiTech University - Shanghai Institute for Advanced Immunological Studies, Shanghai, China, 4Department of Medicine, DIMEDE, Surgical Pathology and Cytopathology Unit, University of Padua, Padova, Italy, 5Istituto Oncologico Veneto, IOV-IRCCS, Padova, Italy.

The recruitment of defined immune cell subsets within the tumoral microenvironment significantly influences cancer cell response. A strong lymphocytic infiltrate associates with good clinical outcome in different human tumors. On the contrary, high frequency of myeloid cells is tightly connected with tumor promotion, metastasis and poor prognosis. Despite the relative profusion of each cell type, the predominant cytokine and chemokine milieu within the tumor microenvironment importantly tips the balance in favor of either anti-tumor immune responses or tumor-promoting responses. In this study, we report the overexpression of the majority of chronic inflammatory diseases including cancer. Reactive Nitrogen Species (RNS) influence homeostatic properties of several proteins at post-translational level. Indeed, the post-translation modifications (PTM) of proteins represent an important level of regulation that must be deeply investigated in cancer. Our group showed that PTM alter the recruitment of distinct immune subsets within tumor primary lesion thus affecting the efficacy of cancer therapy. We originally focused our analysis on CCL2, though other cytokines and chemokines should be target of such modifications and contribute to shape the immune response. One of the possible candidates is the granulocyte colony-stimulating factor (GM-CSF), which is an important regulator of inflammation. Its prominent role as immunomodulatory cytokine has been increasingly considered from different studies linking its deregulation to chronic inflammatory diseases and cancer. Our data indicated that RNS impact on the molecular dynamics and functions of this key cytokine by altering the immune landscape in tumor-bearing hosts.

P.B1.03.13
Direct and indirect effects of various cytokine pretreatment of human peripheral blood mononuclear cells on the proliferation of cervical cancer cells in coculture

G. M. Mujtaba, T. R. Simpson;
Florida Gulf Coast University, Fort Myers, United States.

Immunotherapies are increasingly being developed to target certain tumors and cancers. Here, we examine the direct and indirect effects of various cytokine pretreatment of human peripheral blood mononuclear cells (HPBMC) on the proliferation of cervical cancer cells. HPBMC were pretreated with various cytokines, such as IL-12, IL-15, IL-18, and IFNy, either alone or in combination for 48 hours, after which cells were washed. Washed cells were then either incubated directly with HeLa cells or alone for another 48 hours and their supernatants collected and incubated with HeLa cells. After 48 hours of coculture of HeLa cells with either HPBMC or supernatants, cellular proliferation was measured using the WST-1 proliferation assay kit and absorbance was read at 490nm on a microplate reader. Results show that HeLa cells inhibition required direct contact by HPBMC when cytokine pretreatments involved IL-12 and IL-15. Direct coculture inhibition of proliferation was approximately 54% for IL-12 and 62% for IL-15 pretreatments. On the contrary, IL-18 pre-treated HPBMC cell supernatants had a greater effect on HeLa cell inhibition (40%) than HPBMC directly cocultured with HeLa cells (20%). Both IFNy pretreated HPBMC (33% inhibition) and their resultant supernatants (23% inhibition) had inhibitory effects on HeLa cell proliferation. At optimal inhibitory concentrations, above cytokine combination pretreatments involved IL-12 and IL-15, no additive or synergistic inhibition of HeLa cell proliferation in the coculture assay. Thus, data from this study increases our understanding of the tumor microenvironment and the effect of certain cytokines on peripheral blood mononuclear cell activity on cancer cells.

P.B1.03.15
Porcine Circovirus Type 2 ORF3 protein induces apoptosis in melanoma cells

M. Teras1, A. Rump1, V. Paolme2, S. Rüütel Boudinot3;
1North Estonia Medical Centre, Oncology, Estonia, 2Tallinn University of Technology, Department of Chemistry and Biotechnology, Estonia.

Background. The current treatment of malignant melanoma is limited by the lack of effective therapeutic approaches, and alternative treatments are needed. Proliferative melanoma cells, their neoplastic transformation and other cancers may be treated by virally-encoded apoptotic proteins that are targeted to rapidly multiplying cells. Methods. In the current study, the Porcine circovirus type 2 (PCV2), proapoptotic protein ORF3 was expressed in mouse and human cancer cell lines, and its apoptotic activity assessed. Results. Quantitative assessment of the apoptotic assay by flow cytometry showed that apoptotic cell death was significantly increased in ORF3-expressing malignant cells, compared with ORF3 non-expressing cells. Our data show that PCV2 ORF3 induces apoptosis likely in a caspase-independent manner. ORF3 expression causes an increase in abnormal mitosis in B16F10 melanoma cells by interacting with centrosomes and thereby disrupting formation of the mitotic spindle. In addition, we show that ORF3 of PCV2 also exhibits significant anti-tumor effects in vivo. Although the expression of regulator of G protein (RGS)-16 by recipient mice inhibited the development of grafted melanoma in vivo, it was not required for the antitumoral activity of ORF3. Conclusion. PCV2 ORF3 causes non-bipolar mitosis in rapidly dividing cells and increases the apoptosis of cancer cells. Apoptin might therefore be considered to develop future antitumoral strategies.

P.B1.03.16
Cortisol determines the capability of human acute myeloid leukemia cells to escape immune surveillance via upregulation of Iatrophilin expression on genomic level

S. S. Sakhnevych1, I. M. Yasinska2, A. M. Bratt3, D. Berlouer4, W. Fiedler5, V. Uschkarov5, V. Sumdave5;
1Medway School of Pharmacy, Chatham Maritime, United Kingdom, 2University Hospital Hamburg-Eppendorf, Hamburg, Germany.

Introduction. Acute myeloid leukemia (AML) is a blood and bone marrow cancer, which rapidly develops into a systemic malignancy due to capability of cancer cells to disable anti-cancer immunity. One of the biochemical mechanisms lying in the core of this process is highly upregulated secretion of galectin-9, a tandem protein, which triggers biochemical inactivation of natural killer (NK) cells and killing of cytotoxic T cells. Galectin-9 secretion is mediated by Iatrophilin 1 (LPH1N1), a G-coupled receptor expressed by AML cells but not healthy leukocytes. However, the biochemical machinery which controls these events remains unclear and thus was the aim of this study. Methodology. Primary human AML cells, healthy leukocytes and blood plasma from respective donors as well as THP-1 human leukaocytes and line were used to conduct the work. Western blot analysis, ELISA and qRT-PCR were employed as main research instrumental. Results and Discussion. Cortisol has significantly upregulated LPHN1 expression in AML cells, but not in primary healthy leukocytes. Importantly, cortisol levels were highly upregulated in blood plasma of AML patients compared to healthy donors. Natural LPHN1 ligand, FLRT3 protein present in human blood plasma in its soluble form was found to facilitate galectin-9 exocytosis in AML cells. Conclusion. Our results suggest, that human steroid hormone cortisol normally responsible for regulation of metabolism is used by malignant AML cells to gain capability to disable anti-cancer immunity. In blood plasma of AML patients glucose levels are decreased, which induces biochemical triggering of upregulated cortisol production by hypothalamus and pituitary gland.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

221
Adoptive T cell transfer combined with DC vaccination in patients with metastatic melanoma


1Karolinska Institutet, Stockholm, Sweden, 2Karelska Universitetets sjukhuset, Stockholm, Sweden, 3German Cancer Research Center, Heidelberg, Germany.

Adaptive T cell transfer (ACT) has been reported to induce clinical responses in up to 70% of stage IV melanoma patients. The aim of the MATO2 trial is to investigate the effect of adoptive transfer of autologous, tumor infiltrating lymphocytes (TIL) with or without autologous dendritic cell (DC) vaccination in patients with advanced melanoma. The anti-tumor response will be evaluated and the transferred TILs will be characterized in regard to phenotype and function.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Recently, blocking of the negative checkpoint regulators Programmed Death-1 (PD-1) and Cytotoxic T Lymphocyte Antigen-4 (CTLA-4) showed promising results in the treatment of cancer, highlighting the potential of CD4+ T cells as a tool for cancer immunotherapy.

**Conclusion:** Our results indicate that the addition of serum promotes improved CD19 CAR T cell expansion and viability in vitro. Different culture media promoted different phenotypes of CD19 CAR T cells, which warrants further assessment in clinical settings. Overall, culture medium is a key factor that impacts CD19 CAR T cell performance.
**POSTER PRESENTATIONS**

**P.B1.04.06**

Nanostructures as tools for targeting of Adeno-associated viruses to target-expressing cells

A. Eichhoff, T. Eder, F. Haag, S. Adirouch, F. Koch-Nolette; 1Institute of Immunology, UKL, Hamburg, Germany, 2Institute of Medicine and Pharmacy, Inserm U1234, Rouen, France.

Adeno-associated viruses (AAVs) are widely used as vectors in gene and tumor therapy to treat various diseases. A limiting factor for successful gene delivery without side effects is the broad tropism of AAV serotypes, i.e. the parallel infection of several tissues and cell types. Here, we show that Nanostructures - the single binding domain of camelid heavy chain antibodies - can be used as ligands to target AAVs to specific cells. Nanostructures provide high specificity and stability, and their small size allows easy reformatting as fusion proteins. The ectoenzymes CD38 and CD296 and the P2X7 ion channel were evaluated as target receptors for AAVs. In one strategy, the membrane protein-specific nanobody was genetically inserted into an exposed surface loop of the viral capsid protein VP1 of AAV2 (a variant containing two mutations of arginines to alanines that inhibit binding to HSPG). The presentation of the nanobody on the viral capsid resulted in specific transduction of cells expressing the target with GFP-encoding AAVs. As a second strategy, the membrane protein-specific nanobody was genetically fused via a flexible peptide linker to an AAV1/2 dual-reactive nanobody, thereby generating bispecific nanobody-based adaptor proteins. These adaptors strongly and specifically enhanced the transduction of cells expressing CD38, CD296, or P2X7 by both AAV1 and AAV2.

These results provide proof of principle for nanobody-based strategies to enhance the cell specificity of AAVs and provide a basis for new approaches to optimize Adeno-associated viral vectors for gene and tumor therapy.

**P.B1.04.07**

Regulation of the NK cells melanoma cytotoxic cross talk by nanotechnology assisted p53 reactivation

R. Ibáñez, M. Naddor, V. Venturà, L. Izzo, G. Selivanova, D. Pappalardo, E. Carbone; 1Tumor Immunology and Immunopathology Laboratory, Department of Experimental and Clinical Medicine, University Magna Gracia of Catanzaro, Catanzaro, Italy, Catanzaro, Italy, 2Department of Science and Technology, University of Sannio, via dei Mulini 5/A, 82100 Benevento, Italy, Benevento, Italy, 3Department of Health Sciences, University "Magna Gracia" of Catanzaro, Catanzaro, Italy, Catanzaro, Italy, 4Department of Biotechnology and Life Science, University of Insubria, via J. H. Dunant, 3, 21100 Varese - Italy, Varese Italy, Italy, 5Department of Microbiology, Immunology and Cell Biology, Karolinska Institutet, 17177, Stockholm, Sweden, Stockholm, Sweden.

Introduction: PRIMA-MET and RITA are small molecules able to restore p53 function and induce tumor cells apoptosis. We and others demonstrated that these molecules enhance the NK cell-mediated recognition of solid tumors by promoting the expression of NKG2D ligands on tumor cell surface. To improve the pharmacological effect of these compounds on tumor cells, and increase their solubility and transport efficiency in the body fluids, we analyzed a panel of bioconjugates for their capability to form nanoparticles able to deliver a drug cargo inside tumor cell lines. We found that novel designed FITC-conjugated mPEG-

**P.B1.04.08**

**P.B1.04.09**

**P.B1.04.10**

Heavyuman chain antibodies based on CD38-specific nanobodies effectively inhibit tumor growth in a systemic human lymphoma xenograft model


The cell surface ecto-enzyme CD38 is a target for the treatment of hematological malignancies. Nanobodies derived from camelid heavy chain antibodies are highly soluble and can bind to epitopes that are not accessible for conventional antibodies. Other advantages of nanobodies include their better tissue penetration in vivo, and the facile construction of bi- or multi-specific biologicals by genetic fusion (1). We have generated humanized heavy chain antibodies by fusion of CD38-specific nanobodies to the hinge and Fc-domains of wild type and engineered human IgG1. Some of these heavy chain antibodies mediate potent complement dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) of CD38-expressing tumor cell lines in vitro. In vivo some of the heavy chain antibodies reduced the growth of systemic CA46 lymphomas in tumor-bearing SCID mice more effectively than daratumumab, the benchmark conventional human IgG1 in the clinic (1). Bananas P, Hambach J, Koch-Nolette F. 2017. Nanobodies and NanoBody-Based Human Heavy Chain Antibodies As Antitumor Therapeutics. Front Immunol. 8:1603.

**Materials and Methods:** Primary melanoma cell lines were treated with FITC-labeled PRIMA-1MET-loaded nanoparticles and analyzed for the expression of NK activating/inhibitory molecules by FACS.

**Results:** Our preliminary data showed that FITC-nanoparticles loaded with PRIMA-1MET were detected in the melanoma cytoplasm after 6 days of treatment. Moreover, the treatment increased MICA while it reduced the PD-L1 expression on melanoma cells.

A new formulation of FITC-nanoparticles loaded with RITA has been generated.

**Conclusions:** The new formulation of PRIMA-1MET goes into the melanoma cells and change their immune phenotype.
Enforced expression of a constitutively active form of GSK3β as a novel treatment to combat melanoma through facilitation of dendritic cell differentiation and activation

M. Lopez Gonzales1, R. van der Ver1, D. Oosterhoff1, J. Jan Lindenbergh1, H. de Haan1, W. Dong3, W. van Beusechem2, T. D. de Gruj1,
1Cancer Center Amsterdam, Amsterdam, Amsterdam, Netherlands, 2QRC Therapeutics BV, Amsterdam, Netherlands.

Even though immune checkpoint blockade has increased the overall survival of melanoma patients considerably, not all patients respond to this treatment, in part due to a lack of T-cell infiltration in the tumor fields. Activation of the Wnt signaling pathway has been identified as a molecular mechanism underlying this lack of immune infiltration, resulting in a lack of cross-presenting dendritic cells (DCs), which in turn are vital for the chemo-attraction and activation of tumor-infiltrating T-cells. β-catenin levels can be directly regulated by glycogen synthase-3beta (GSK3β), an enzyme involved in various signaling pathways. When GSK3β is activated it will phosphorylate β-catenin which will lead to its inactivation and degradation. Here, we report that modulation of GSK3β at the level of DCs plays a key role in their differentiation and maturation, and that overexpression of the constitutively active form of GSK3β (CA.GSK3β) renders DCs refractory to the suppressive effect of IL-10, an important suppressive factor released by metastatic melanoma, and of melanoma-derived supernatants. Moreover, the inhibition of GSK3β in melanoma cells increases their suppressive effect on monocyte-to-dendritic cell differentiation. Conversely, the enforced over-expression of CA.GSK3β reduces this suppressive effect and drives DC development to a type-1 T-cell-activating phenotype.

Based on our findings, we hypothesize that the enforced expression of CA.GSK3β at the melanoma tumor site may enhance the infiltration and activation of DC and T-cells and thereby render previously resistant tumors sensitive to immune checkpoint blockade.

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Use of alpha technology to identify novel small molecules that specifically inhibit LAG-3 binding to its ligand HLA-DR

G. H. Mason1, B. J. MacEachlan1, A. Greathouse Watson1, F. Tripel1, D. K. Cole1, A. Godkin1.
1Division of Infection & Immunity, Cardiff, United Kingdom, 2ImmuneP, Orsay, France, 3Immunocore Ltd, Abingdon, United Kingdom.

Introduction: CD4+ helper T cells express a series of activating and inhibiting co-receptors. These play a role in both autoimmunity and cancer immunity. Immune checkpoint inhibitors (ICI) which target molecules mediating inhibition (e.g. PD1, CTLA-4) may result in enhanced anti-tumour immunity. Lymphocyte activation gene-3 (LAG-3) is a negative regulator of T-cells that has not so far been successfully targeted by monoclonal antibodies. LAG-3+ T-cells within tumours have been shown to be immunosuppressive and are associated with poor prognosis. As an alternative to expensive antibody based therapies, we conducted a small molecule library screen to identify lead compounds which may interfere with the interaction of LAG-3 with its ligand HLA class II.

Materials and Methods: Alpha technology, a bead based assay for the detection of protein-protein interactions (PPI), was used to screen a 50,000 compound library against LAG-3+ HLA class II receptor control PPI using-TCH PHA.

Results: 50 compounds which specifically block the interaction between LAG-3 and HLA II have been identified as true assay hits which block the LAG-3-HLA II interaction without blocking an irrelevant PPI.

Conclusions: Having identified hit compounds, these compounds are being tested using a LAG-3 expressing reporter cell line. Generation of a novel LAG-3-HLA II inhibitor, will enable us to learn more about the role of LAG-3 in disease setting, and aid the development of novel therapeutics.

B-cell lineage-specific transcription coactivator BOB1 is indispensable for multiple myeloma cell survival and allows for superior TCR-based targeted therapy

R. M. Reijmers1, A. D. Meringa, L. Jahn, F. Falkenburg, M. H. Heemskerk;
Leiden University Medical Center, Leiden, Netherlands.

Although still incurable, much progress has been made in the treatment of multiple myeloma (MM). Recent advances in immunotherapy have contributed substantially, of which the most promising, T cells modified to express a chimeric antigen receptor (CAR) directed against B cell maturation antigen (BCMA). Another approach is introducing a transgenic T cell receptor (TCR) into cytotoxic T cells. Recently, for MM, we successfully demonstrated in vivo efficacy of a transgenic TCR targeting the transcription coactivator octamer binding protein-1 (BOB1) in the context of HLA-A*0207.02. BOB1 is a B cell lineage specific protein that is highly expressed in all B cell malignancies, including MM. Like rituximab (anti-CD20) treatment, targeting BOB1 will only affect the B cell lineage, which makes it attractive for immunotherapy with high on-target and low off-tumor effects. This prompted us to further explore the significance of BOB1 in MM. To this end, we applied CRISPR/Cas9 to disrupt BOB1 expression in several MM cell lines. Remarkably, upon single-cell sorting and DNA sequencing, all targeted clones revealed in-frame deletions only. We are currently extending these findings to other B cell malignancies and study the functionality of the in-frame mutated BOB1 variants on target gene expression, and study the effect on in vitro and in vivo growth. Together, these data suggest that BOB1 is indispensable for myeloma cell survival, which identifies BOB1 as a superior target for TCR-based immunotherapy. This work was financially supported by Bellicum Pharmaceauticals.

Identifying immunological properties of natural products for the treatment of chemoresistant tumors and bacterial pathogens

L. Richter1, M. Franken1, P. Proksch1, S. Scheu1;
1Heinrich Heine University, Institute of Medical Microbiology and Hospital Hygiene, Duesseldorf, Germany, 2Heinrich Heine University, Institute of Pharmaceutical Biology and Biotechnology, Duesseldorf, Germany.

Introduction: The most abundant human diseases worldwide include cancer and bacterial infections. To overcome the development of resistances against cytostatics and antibiotics, there is a rising need to find new drugs. Natural products comprise many untested highly bioactive molecules inspiring medical research. We are looking for compounds that modulate immune effector functions and additionally target tumors and pathogens, thus reducing the risk of resistances. Material and methods: A library with 240 natural products derived from endophytic fungi and marine sponges undergoes different screenings to determine promising compounds. After having completed cytotoxicity screenings, we are currently using an IL-12p40 fluorescence reporter mouse line to test selected natural products for their ability to induce or enhance expression of IL-12 in macrophages or dendritic cells via flow cytometry and ELISA. The thus identified immune activating compounds will be further investigated in T cell activation assays for their potential to promote T cell priming by dendritic cells. Results: We have identified 41 natural products that are non-toxic to immune cells but simultaneously toxic to tumor cells or pathogens. For application of the aforementioned IL-12p40 assay, cell culture and FACS staining conditions have been successfully established and multifunctional natural products will be screened for immune activation. Conclusions: Promising multifunctional, immune activating natural products will further be biochemically optimized for immune modulatory effectivity and tested in in vivo tumor and infection mouse models. This work is funded by the German Research Foundation (DFG).

Human γT cells: Presentation Of Tumour Antigens And Induction Of Anti-Tumour Immunity

T. Rus1, M. Ebert1, B. Moser1,2;
1Cardiff university, School of medicine, Cardiff, United Kingdom, 2Systems Immunity Research Institute, Cardiff, United Kingdom.

An important discovery that activated human blood γT cells behave as professional antigen-presenting cells (γT-APCs) has highlighted their potential use as cellular vaccines. γT-APCs are ideal candidate for immunotherapy as they are non-MHC-restricted and they do not mount unwanted/unpredicted cross-reactivities in patients. They are capable of inducing potent antigen-specific and MHC-restricted responses that may boost anti-tumour cytotoxic T cell responses. Lastly, they can be generated in large numbers (>10^{10}) in vitro from small blood samples and predominantly secrete proinflammatory cytokines (IFNγ, TNFα), which may help overcome immune inhibitory conditions frequently associated with tumours. It has been shown that γT-APCs can excellently process microbial antigens (influenza, CMV, Mtb), including peptides, whole proteins and cell extracts, and induce strong antigen-specific and MHC-restricted responses that may boost anti-tumour cytotoxic T cell responses. Lastly, they can be generated in large numbers (>10^{10}) from small blood samples and predominantly secrete proinflammatory cytokines (IFNγ, TNFα), which may help overcome immune inhibitory conditions frequently associated with tumours. It has been shown that γT-APCs can excellently process microbial antigens (influenza, CMV, Mtb), including peptides, whole proteins and cell extracts, and induce strong antigen-specific and MHC-restricted responses that may boost anti-tumour cytotoxic T cell responses.
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PD-1 blockade impairs the anti-tumor activity of innate immune cells stimulated with TLR9 agonist
C. Storl1, M. Sammariva1, M. De Cesare2, E. Tagliabue1, A. Balsari1, L. Sfondrini1
1Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milan, Italy, 2Molecular Pharmacology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, 3Molecular Targeting Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

Introduction: TLRs agonists are known to activate innate immune cells and can exert anti-tumor activity. We previously reported that locoregional administration of CpG-ODN, synthetic TLR9 agonist, inhibited IgG1 anti-human ovarian cancer cell growth in athymic nude mice, preclinical model devoid of T lymphocytes in which anti-tumor immune response is mediated only by innate immune system. PD-1 receptor is expressed by activated T lymphocytes and also by innate immune cells. Interaction of PD-1 with PD-L1 or -2 blunts immune response, but specific antibodies blocking PD-1 can re-activate it. Therefore, combinations of TLRs therapies with anti-PD-1 antibody may be a promising new therapeutic strategy.

Methods: Nude mice were intraperitoneally xenografted with IGROV-1 cells and locally treated with CpG-ODN in combination with anti-PD-1 antibody. Macrophages were depleted by liposomes-containing clodronate. Immunocompetent mice injected with B16 melanoma and 4T1 breast cancer cells were treated as above.

Results: CpG-ODN/anti-PD-1 antibody combination in IGROV-1-injected mice was found to be less efficacious than CpG-ODN alone, independently from the treatment schedules and anti-PD-1 clone used. The same combination in B16- or 4T1-injected immunocompetent mice did not reveal a similar effect. Administration of anti-PD-1 antibody immediately after IGROV-1 tumor injection determined tumor growth acceleration. Immunohistochemical analysis showed an increase of Arg1+ intratumoral macrophages in anti-PD-1 treated group. Macrophage depletion restored CpG-ODN antitumor effect when combined with anti-PD-1 antibody.

Conclusion: Our results suggest that blocking PD-1 pathway reduced the therapeutic efficacy of CpG-ODN probably due to an effect probably mediated by macrophages.

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P.B1.04.17
Estimation of antitumor effect of chemoinmunotherapy with methotrexate nanconjunctates and dendritic cell-based vaccines in MC38 murine colon carcinoma model
S. Szczygieł1, N. Anger2, K. Wegierek3, M. Mierzejewska3, J. Rossowska3, T. Gószczyński3, M. Switalska3, E. Pajtasz-Plasecka3; Ludwig Hersfeldt Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.

Nanconjunctates of methotrexate (MTX) and hydroxyethyl starch (HES) are a new type of therapeutic compounds formed from certified drugs widely used in medicine. Conjugation of MTX with HES grafts onto the half-life of MTX and enhances its side effects in order to enhance the therapeutic effect, we combined MTX-HES nanconjunctate with bone marrow-derived dendritic cells (BM-DCs), which are considered to be capable of activating the immune system in the host.

To determine the antitumor effect of chemoinmunotherapy, mice with subcutaneously growing MC38 tumor intraorally received MTX or MTX-HES and three days later, intratumorally injected pentamural injections of mature BM-DCs. To evaluate the changes in percentage of tumor infiltrating lymphocytes and myeloid cells, three days after chemotherapy or seven days after immunotherapy, tumors were collected for multiparametric flow cytometry analyses.

On the 3rd day after chemotherapy in MTX-HES-group the highest percentage of CD4+ and CD8+ T cells infiltrating tumor tissue was observed, moreover in both groups the percentage of Tregs was drastically decreased. SUPPLEMENTARY WITH BM-DC-vaccines intensified this effect, additionally an increase in the percentage of NK cells was also noticed. Infiltration of leukocytes was enhanced only after chemoinmunotherapy, moreover application of BM-DC-vaccines resulted in reduction of percentage of myeloid cells with suppressor activity.

Concluding, therapy with nanconjunctates and BM-DCs-vaccines resulted in efficient response against growing tumor. These findings demonstrate that methotrexate-nanconjunctate immunomodulates the antitumor effect and this benefit was effective for generation of proper immune response by dendritic cells.

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P.B1.04.18
Sodium stibogluconate in conjugation with CD47-SIRPα checkpoint blockade enables rituximab-mediated killing of B lymphoma cells by neutrophils
D. J. van Rees1, M. van Houdt1, A. Toaf2, P. Verkuiljen3, K. Schramoel4, S. Frenke5, T. W. Kuipers6, T. K. van den Berg7, H. H. Matlung6;
1Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, 2Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam, 3Department of Molecular Cell Biology and Immunology, VU medical center, Amsterdam, Netherlands.

Rituximab (Rmab) is used as a first-line treatment for CD20+ B-cell malignancies. It is believed to act by a combination of direct and immune-mediated effects, including complement- and immune cell-dependent mechanisms. However, neutrophils, the most abundant effector cells mediating antibody-dependent cellular cytotoxicity (ADCC) are incapable of killing Rmab-opsonized B lymphoma cells. Instead, Rmab triggers neutrophil trogocytosis of CD20-containing plasma membrane fragments of the target cells, which is mediated only by innate immune system. PD-1 receptor is expressed by activated T lymphocytes and also by innate immune cells. Interaction of PD-1 with PD-L1 or -2 blunts immune response, but specific antibodies blocking PD-1 can re-activate it. Therefore, combinations of TLRs therapies with anti-PD-1 antibody may be a promising new therapeutic strategy.

Sodium stibogluconate in conjunction with CD47-SIRPα checkpoint blockade enables rituximab-mediated killing of B lymphoma cells by neutrophils

Supported by AIRC

P.B1.04.19
The supernatant of immature dendritic cells mediates RIP1-dependent apoptosis
Z. Varga1, E. Jakab-Racz, A. Szabo, E. Rajnayová, G. Koncz1; Department of Immunology, University of Debrecen, Debrecen, Hungary.

Dendritic cells (DCs) are known to engulf dead cells continuously and present antigenic fragments derived from infected cells and tumor antigens thus having the capacity of triggering naive CD8+ T cells. The cross-priming potential of DCs has been known as a unique route to initiate classical T cell responses. In vitro generated moDCs have also been demonstrated to induce apoptosis in target cells. We provide evidence here that supernatants of activated immature moDCs activated by PRR agonist (LPS, poly (I:C) or CI-075) induces cell death on Jurkat cells. In contrast, the supernatant of mature DCs were less cytotoxic, which may effect on the adoptive DC therapies. TNF-β fusion protein inhibited the cytotoxicity activity of DCs in contrary to Fas and TRAIL antagonists, suggesting it is a TNF dependent, but Fas and TRAIL independent process. In contrary to cytotoxic T cell-mediated killing, secreted vesicles from culture supernatants of moDC were not able to generate immunological mechanisms of DC-mediated signaling we were able to detect RIP1-dependent cell death. Pretreatment of target cells with a pan caspase inhibitor (zVAD) completely blocked moDC triggered apoptosis, but necroptosis inhibitor ( nec-1) did not prevent this cell death.

In summary, our results indicate that the supernatant of immature dendritic cells induces RIP1-dependent apoptosis, which may be relevant for tumor immunotherapy broadens the plethora of cytotoxic mechanisms acting against tumor cells.

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P.B1.05.01
Tumor vaccination principles and Immunotherapy - Part 5

From mono- to bivalent: increasing affinity of EGFR-specific target modules results in enhanced anti-tumor properties of the UniCAR system
S. Albert1, C. Amidt2, S. Koristka3, N. Berndt2, R. Bergmann2, A. Feldmann2, M. Bachmann1;1University Cancer Center Dresden, Dresden, Germany, 2Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Institute of Radiopeharmaceutical Cancer Research, Dresden, Germany, 3German Cancer Consortium (DKTK), partner site Dresden and German Cancer Research Center (DKFZ), Heidelberg, Germany.

Introduction: Despite introduction of conventional cancer therapies, treatment of epithelial tumors needs to be further optimized. Especially chimeric antigen receptor (CAR)- modified T cells possess a tremendous immunotherapeutic potential. However, due to T cells central control mechanisms, we established the switchable modular UniCAR system. This system is composed of two distinct components, T cells expressing a universal CAR (UniCAR) and exchangeable tumor-specific target modules (TMs). Based on the modular structure, engrafted T cells are inert in the absence of TMs and only switched on in their presence.

Materials and Methods: For redirection of UniCAR T cells to epithelial tumors, we recently generated a monovalent nanobody-based e-EGFR TM. After expression in CHO cells, the construct immunized and antigen-specific tumor cell lysis. To analyze whether the functionality can be improved by increasing the affinity, a bivalent e-EGFR-e-EGFR TM was established and compared to the monovalent counterpart.

Results: Due to raising the number of binding sites, the bivalent TM shows an increased avidity, higher levels of released pro-inflammatory cytokines and an improved killing capability in vitro and in vivo. Nevertheless, cells with an EGFR density comparable to physiological numbers of EGFR are not eliminated and, therefore, destruction of healthy tissues is rather unlikely. Conclusions: Summing up, increasing the avidity of the TM enhances its functionality.
Regulatory T cells (Tregs) are important contributors to immunosuppression in tumors. Based on our previous work, we believe that TGF-β1 is a major player in Treg-mediated immunosuppression, which could thus serve as drugs to increase anti-tumor immune responses. Our blocking antibodies bind human GARP, but not m(urine) GARP. We thus generated mice expressing a humanized version of Garp (mGarpHu137-139 mice) that can be bound by the blocking anti-hGARP antibodies.

In this project, our main objective is to assess the activity and toxicity of blocking anti-hGARP antibodies in preclinical models of cancer in mGarpHu137-139 mice.

Generation of mGarpHu137-139 mice was performed by subcontractors in two genetic backgrounds, namely C57BL/6 and DBA/2. Homozygous mutant mice were born to Mendelian ratios from heterozygous couples, and showed no gross anomaly after birth. We show that the mGarpHu137-139 mutation does not affect the expression pattern of the Garp gene in the genetically manipulated mice, by comparison to wild-type littermates. Flow cytometry and qPCR analysis of various organs (thymus, blood, spleens, bone marrows, lymph nodes) indicate normal hematopoietic cell development and numbers. No signs of auto-immunity or general T cell activation is observed in mutant mice. We verified that from mutant mGarpHu137-139 mice were able to produce active TGF-β1 upon in vitro stimulation, and that this production could be blocked with the blocking anti-hGARP antibodies.

Towards a clinical grade CD137-based isolation and expansion of neoantigen-specific T-cells for tailored cancer adoptive cell therapies

V. Bianchi, S. Bobisse, R. Genolet, L. Kandolf, G. Coukos, A. Harari; Ludwig Institute for Cancer Research, Lausanne, Switzerland.

Mounting evidence suggests that neoantgens tumor neo-antgens derived from non-synonymous somatic mutations represent ideal candidates to target with T-cell mediated immunotherapies. The selective isoform of low frequency neoantigen-specific T-cells in patients’ TIL (Tumor Infiltrating Lymphocyte) and PBMC (Peripheral Blood Mononuclear Cell) bulk populations represents one of the major challenges in the development of personally tailored cancer T-cell therapies. A surface marker uniformly upregulated upon activation would facilitate the isolation of neoantigen-specific T-cells when cognate peptide-HLA multimers are not. The TNFR family member CD137 has been well characterized as a specific marker of TCR-induced activation of conventional CD8+ (and CD4+)-T-cells. Following antigen-specific stimulation, CD137 expression on CD8+ T-cells peaks at 20-24h, thereby allowing for the detection of viable T-cells displaying reactivity to the epitope of interest. Within the framework of our translational neoantigen discovery platform, we are in the process of developing a clinical grade strategy for the ex vivo isolation and expansion of viable neoantigen-specific T-cells based on CD137 expression and flow-cytometric separation from patients’ TIL and/or blood samples. The protocol is currently being validated on healthy donors PBMC samples with known viral and tumor T-cell reactivities and, in close collaboration with the Process Development team at CTE (Lausanne), will be performed with clinical grade compliant materials and reagents. By enriching for neoantigen-specific T-cells prior to infection, it may be possible to improve the overall response rate achieved so far by unselected adoptive T-cell therapies.

Preclinical Safety, Pharmacokinetics and Pharmacodynamics of BION-1301, a first-in-class antibody Targeting APRIL for the Treatment of Multiple Myeloma

J. Dulos; Aduro Biotech Europe, Oss, Netherlands.

BION-1301 is a first-in-class humanized antibody targeting APRIL (TNFSF13). A single-dose non-human primate (NHP) study administering intravenous BION-1301at 0.3, 3 and 30 mg/kg dose levels yielded PK parameters typical for IgG4 class antibodies and an absence of tolerability issues. PD analysis showed a statistically significant reduction in total IgA and IgM in a dose-dependent fashion. Consistent with previous observations in hAPRIL transgenic mice, BION-1301 reduced TNF-specific IgA and IgM in NHP in a dose-dependent fashion. Material and methods: We first evaluated the O-acetyl-GD2 (OacGD2) expression on GSC by flow cytometry. Next, we characterized the effects of anti-OacGD2 monocular antibody 8B6 + TMZ combination on tumor cell viability, using an MTT assay and in vivo in an orthotopic GBM xenograft mouse model.

Results: Here we found that OacGD2 ganglioside is expressed on GSC. We further demonstrated that 8B6 + TMZ synergistically inhibited glioblastoma cell proliferation and compromised GSC survival in vitro. These findings correlated with longer therapeutic response evidence in vivo. Mechanistically, we evidenced that 8B6 oncosis-like properties increased cytotoxic drug uptake into GMB cells. As a result, 8B6 + TMZ combination induced significantly increased DNA damages and tumor cell death than either 8B6 or TMZ monotherapy.

Conclusion: Taken together, our data provides a mechanistic rationale for anti-OacGD2 monoclonal antibodies as chemo-sensitizing agents to improve the response of GBM to TMZ. Disclosure: JF, SFa and SB are designed as inventor of pending patents covering cancer immunotherapy targeting O-acetyl-GD2.
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P.B1.05.07
Depletion of CAR-expressing lymphocytes using autologous anti-CAR-engrafted T cells
S. Koriatka, F. Ziller-Walter, A. Feldmann, C. Arndt, S. Albert, G. Ehninger1,2,3, M. Bornhäuser1,2, M. Bachmann1,2,4
1Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Dresden, Germany; 2Institute of Cancer Immunology, University of Freiburg, Germany; 3Carl Gustav Carus’ Technische Universität Dresden, Dresden, Germany; 4Department of Oncology, University Hospital, Dresden, Germany

Adaptive transfer of chimeric antigen receptor (CAR) T cells represents one of the fastest growing areas in cancer immunotherapy. Albeit gene-modified cells have demonstrated unparalleled antitumor efficiency in B cell malignancies, highly potent CAR T cells can cause severe and partly life-threatening side effects including cytokine release syndrome, neurological toxicity and off-target effects. Hence, there is an increasing demand for developing effective approaches to selectively ablate gene-modified cells in vivo. Previously, we described an epitope tag (E-tag) derived from the human nuclear protein Lq that is incorporated into the extracellular domain of CARs and accessible by an anti-Lq monoclonal antibody (mAb). Based on this mAb, we generated a novel CAR construct for specific binding and depletion of E-tag-expressing CAR T cells.
We demonstrate that anti-E-tag-redirected T cells selectively eliminate CAR T cells that extracellularly express the E-tag whilst CAR T cells lacking this tag are not attacked. Interestingly, T cell killing is reciprocal and occurs in dependence of an intracellular signaling domain. Our studies further indicate that T cells expressing high CAR levels are more efficiently depleted than T cells with low CAR expression. Besides, CD4- and CD8- target cells are equally well eliminated by both CD4- and CD8- effector T cells. Overall, we provide an approach for specific and efficient depletion of on-target CAR T cells in case patients experience severe side effects. The E-tag can be incorporated into all CARs of the targeted tumor antigen and represents a promising tool to improve safety of cell-based immunotherapies.

P.B1.05.08
Peripheral blood immune profiling to predict response to PD-1 checkpoint blockade in patients with advanced non-small cell lung cancer
S. Lopez-Lastra1, T. Le Bourgeois2, F. Bidard3,4, S. Amigorena4, D. Lantz5, E. Ramano6,7
1Center of Cancer Immunotherapy, Institut Curie, Paris, France; 2Pitié-Salpêtrière University Hospital, Paris, France; 3INSERM U932, PSL Research University, Institut Curie, Paris & St. Cloud, France; 4Department of Oncology, Institut Curie, Paris & St. Cloud, France; 5Université Versailles Saint-Quentin-en-Yvelines & Université Paris-Saclay, Saint Cloud, France

Introduction: Immune checkpoint inhibitors have naturally the remarkable history of good clinical results in non small cell lung cancer (NSCLC), with improved clinical responses and increased survival compared to standard therapy. However, over 80% of unselected NSCLC patients do not respond, highlighting the need of theranostic biomarker discovery.
In a cohort of NSCLC patients treated with anti-PD-1 blockade, we investigated blood immune parameters at baseline and patient characteristics as potential theranostic biomarkers.
Methods: Thirty-four patients with locally advanced/metastatic NSCLC received either nivolumab or pembrolizumab as ≥ 2 line treatment in a prospective study at the University Hospital of Tübingen. Peripheral blood mononuclear cells were analyzed at baseline and correlated with outcome parameters based on immune-related RECIST criteria.
Results: Baseline CD3+/CD14+ ratio was the strongest predictive biomarker with patients achieving progression free survival (PFS) ≥ 12 months showing an average ratio of 1.91 vs 1.11 in patients with PFS ≤ 6 months (p=0.003). We found a strong positive correlation between the proportion of HLA-DR+CD14+monocytes and the PFS (r=0.471), with objective responders showing higher CD168 expression, suggesting an improved antigen-presenting capacity. In addition, patients with a PFS ≥ 24 months, displayed higher proportions of CD8+ T cells as compared to patients with PFS ≥ 6 months. Other cytokines such as IL-10, IL-6 or IFN-γ were not correlated with outcome. Based on these results, we conclude that the association of baseline serum albumin with clinical outcome in NSCLC, with levels ≥ 3.9 g/dl significantly associated with improved PFS (p=0.026).
Conclusions: Our study identifies promising, immune-related, theranostic biomarkers in NSCLC patients treated with PD-1 blockade.

P.B1.05.09
Dual CAR antiHER2-CD137 and anti-MUC1-CD3: a proposal of immunotherapy against anti-HER2 breast cancer
B. Marzal, S. Betriu-Mendez, V. Ortiz-Maldonado, A. Boronat, J. Yagüe, M. Juan, A. Pratt
1Hospital Clinic - Servei d’ImmunoOncology, Barcelona, Spain; 2Hospital Clinic - Servei d’Hematologia, IMHCMO, Barcelona, Spain; 3Plataforma d’Immunoteràpia HClinic-HSID, Barcelona, Spain; 4Hospital Clinic - Servei d’Oncologia, IMHCMO, Barcelona, Spain

Introduction: Chimeric antigen receptors (CARs) are a new promising tool to overcome immunotherapy deficits. Despite great results in the treatment of hematologic malignancies, in solid tumors there are still some great challenges to overcome, being to avoid on-target off-tumor effects one of the main aspects to improve. In our project we propose a novel dual CAR for the treatment of HER2+ breast cancer targeting ErbB2 (HER2) and Mucin-1 (MUC1). We present here some preclinical steps of this proposal.
Methodology and Results: After synthesizing scFv-HER2 and scFv-MUC1, we construct individuals CARs (CAR-HER2 and CAR-MUC1) with scFv sequence and 4-1BB/CD3i triple stimulation domains. Delivering them into human T cells by lentiviral transduction for each CAR, we tested their specific and powerful. Dual CAR was also synthesized dissociating co-stimulatory domains: scFv-HER2 with 4-1BB and scFv-MUC1 with CD3i. We evaluate the specificity, functionality, and safety by comparing cytotoxicity in co-culturing with SK-BR3 and T-47D breast cancer cell lines.
Conclusions: The creation of a dual CAR to fight against breast cancer HER2+ is feasible and it is a new promising proposal for the treatment of HER2+ breast cancer tumors.

P.B1.05.10
A high throughput approach for the parallel identification of TCRs recognizing multiple antigens with clinical relevance for the treatment of B cell malignancies

CAR-T cell therapies for the treatment of B cell malignancies have shown great promise in clinical trials. However, antigen negative escape variants can cause disease relapse and therapy resistance. To target new targets we propose a TCR-based approach. To target both intracellular and extracellular proteins. Illumina HT-12 microarray data was used to select 31 target genes expressed in B-cell malignancies but not in healthy tissues other than B-cells. To broaden to scope of TCR gene discovery, the Institut Curie. Peripheral blood mononuclear cells and serum were analyzed at baseline and correlated with outcome parameters based on immune-related RECIST criteria.

Conclusions: The creation of a dual CAR to fight against breast cancer HER2+ is feasible and it is a new promising proposal for the treatment of HER2+ breast cancer tumors.

P.B1.05.11
Hi-mov 1: A high throughput cell-cell avidity screening and sorting acoustic force based technology
W. Schepers1, E. Merino Rodríguez2, R. Braster1, G. Sitter1, F. Oswald1, B. Dressier1, T. Schumacher1, A. Candel1
1The Netherlands Cancer Institute, Amsterdam, Netherlands; 2LUMICKS, Amsterdam, Netherlands

Adaptive cell therapy can be an effective treatment option in a proportion of cancer patients. Nevertheless, the clinical efficacy of such therapies is likely limited by a lack of tools to quantitatively sort and select the most potent tumor-reactive immune cells at high throughput. Here, we developed a novel platform that uses acoustic force manipulation to query the interaction strengths of tumor-specific T cells with their cognate binding partners. This technology, based on acoustic forces, is a lab-on-a-chip assay that allows the assessment of T-cell/antigen interactions in parallel. Moreover, this technology provides an accurate, label-free method to isolate cells based on their avidity to specific targets, such as (tumor) cells as well as proteins, peptides, and viruses. As a proof of concept, we validated our technology by analyzing the functional avidity of T-cells towards tumor cells and found that it permits the separation of tumor-specific T-cells from non-specific bystander T-cells. These data demonstrate the potential of this platform in profiling T-cell tumor-cell interaction and pave the way to quantitative cell-cell avidity studies as well as a selection of patient-specific immune effector cells or immune receptors for therapeutic use.

P.B1.05.12
HLA DP as a transmembrane anchor for chimeric TCR dimers to improve TCR transgenic therapies

Ex vivo introduction of tumour-reactive T-cell receptor (TCR) constructs into patient-derived T-cells result in redirected T-cell responses towards tumours. TCRβ expression depends on the formation of the CD3 signalling complex via non-covalent interactions within the transmembrane (TM) domains. Competition between endogenous TCR and introduced TCR for binding to the CD3 signalling complex has been shown to interfere with introduced TCR functionality and reducing future therapeutic efficacy. To overcome competition, chimeric TCR dimers (iTCD) were designed to incorporate TM domains from i) heterodimeric TM proteins, linked to the intracellular signalling domains of CD3ζ.

228
We hypothesised that CD3δε would demonstrate CD3-δε-independent expression of induced TCR and allow for improved antigen-specific T-cell responses against tumour targets. However, we showed (1) that co-expression of CD3δε with the extracellular domains of TCRαβ, specific for B cell antigen BOB1, 2) the TM domains of HLA-DPβ1 and 3) the intracellular CD3ζ signaling motifs (BOB1-3ζ). Firstly, BOB1-3ζ was expressed in TCRCD3ζγ-Jurkat-76 cell lines and demonstrated BOB1-specific PMHC-tetramer binding at the cell surface. Furthermore, in contrast to wild-type BOB1-TCR, BOB1-3ζ expressing Jurkat-76 cells did not show surface expression of CD3, demonstrating CD3-δε-independent expression of CD3δε. Next, BOB1-3ζ was expressed in primary CD8 T-cells and co-cultured with BOB1-expressing tumour-target cells. Despite demonstrating BOB1-specific PMHC-tetramer binding, BOB1-3ζ expressing CD8 T-cells were unable to produce functional T-cell responses against tumour targets. Inclusion of CD3ζ intracellular signaling motifs also did not promote functional T-cell responses against tumour targets. In conclusion, in the context of the BOB1-specific TCR, HLA-DPζγ appeared to be unsuitable as a TM anchor for functional TCR expression.

P.B1.05.13 Novel method for the manufacture of CAR-T-cells: Effects of cytokines in cell culture in the media on the phenotype of CAR-T-cells
J. Musil, P. Přásková, P. Otahal, P. Gabriel, M. Kroučková, Š. Němčeková; iHBT, Prague, Czech Republic.

Current manufacture of clinical CAR-T-cells is primarily based on lentiviral/retroviral transduction of CD3/CD28 activated T-cells and subsequent cultivation in the presence of IL-2. This well-established approach, however, has few weaknesses such as that it induces polyclonal T-cells activation resulting in low frequency of transduced T-cells and that the anti-CD3/CD28 activation is supra-physiological and modifies the differentiation of T-cells. Recently, alternative methods for efficient T-cells expansion were developed which are based on T-cell stimulation with antigen followed by cultivation in the presence of cytokines IL4 and IL7. We present a novel method of manufacturing CD19+ CAR-T-cell by electroporation of plasmid DNA followed by cultivation in the presence of cytokines IL4, IL7 and/or IL2+1 without any additional artificial T-cell activation step. This method leads to a spontaneous expansion of T-cells probably through recognition of endogenously present B cells, which results in overall CAR+T-cell yield (>90% CAR+) surpassing the standard methods. Cultivation of CAR-T 9 T cells in the presence of IL4 and IL7 preferentially expands CD4+ CAR T cells. Addition of IL2 or IL21 into this cytokine cocktail enhances expansion of CD8+ CAR T cells. However, IL21 preferentially expands CD8+ central memory T-cells. Furthermore, it enhances expression of co-inhibitory molecules CD28 and CD70 on CD8+ CAR-T Cells and decreases expression of inhibitory receptors. This method is supported by grant NV15-34498A of the Ministry of Health, Czech Republic and by the European Regional Development Fund and OP RDE, Ministry of Education, Youth and Sports of the Czech Republic (project AIHHP: CZ.02.1.01/0.0/0.0/16_025/0007428).

P.B1.05.14 Devising a dual chimeric antigen receptor system to prevent on-target off-tumor effect of engineered T cells
T. Peters1, V. Gudipati1, O. Dushek1, M. Hudecek1, J. B. Huppa1; 1Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria, 2Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom, 3Medizinische Klinik und Poliklinik II, Universität Würzburg, Würzburg, Germany.

CAR-T cell therapies have shown to be potent tools in the fight against cancer as validated by the recent FDA approval of two such therapies against B cell malignancies. However, a major hurdle in the use of CAR-T cells is the existence of tumor-specificity due to the non-existence with all current CARs of the antigen for the CAR to act upon. As a consequence, some CAR-T cells attack invariably healthy tissue. To overcome this impediment, we are devising a logic gate CAR-T cell system based on the combinatorial recognition of at least two antigens, which we expect to confer sufficient discriminatory power for cancer therapy with much reduced off-target toxicity. To this end we co-express in addition to the tumor-associated antigen-specific CAR a second CAR with inhibitory properties (iCAR) binding to a surface antigen exclusively present on healthy tissue to be protected from CAR-T cell attack. So far, our system can seamlessly re-engineer the CAR from the well characterized highly affinity 164T4 CAR, which targets HLA-A201/NY-ESO-1 as model antigen and which can be precisely fine-tuned through the use of NY-ESO-1-derived altered peptide ligands within an affinity spectrum covering 6 orders of magnitude. Tumor specificity, killing capacity and intracellular signaling capacities will be assayed through (i) conventional immunological assays and (ii) a preclinical molecular imaging platform, which involves the use of protein-functionalized planar supported lipid bilayers serving as target cell surrogate in combination with advanced microscopy affording single molecule resolution. This project is funded by the Marie Skłodowska-Curie action EN-ACTING program, from the European Commission.

P.B1.05.15 Increasing CAR T-cell efficacy in prostate cancer with an immunocytokine targeted to the tumour microenvironment
E. Runbeck1, J. Maher, S. Papa; King's College London, London, United Kingdom.

Chimeric Antigen Receptor (CAR) T-cell therapy received landmark FDA approvals for haematological indications in 2017. Solid tumours raise many challenges and anticipated breakthroughs remain elusive. Prostate cancer (PCa) is a highly prevalent disease with a significant unmet need in the advanced setting. The Prostate Specific Membrane Antigen (PSMA) CAR P28E has established in vitro and in vivo efficacy in PCA models but has not met expectations in clinical studies highlighting the need for strategies to enhance efficacy. We have co-expressed the chimeric cytokine receptor 4αβ (consisting of the interleukin-4 (IL-4) receptor alpha in series with the IL-2/15 receptor beta), with P28E (4P2). In response to IL-4, 4αβ delivers an IL-2/15 signal in the CAR-T cell. Fibroblast Activation Protein (FAP) is a strongly expressed trans-membrane enzyme found in the stroma of epithelial tumours and healing wounds. We hypothesised that a FAP specific IL-4 immunocytokine would enhance 4P2E proliferation, survival and cytotoxicity in the tumour microenvironment. FAP-specific hybridomas were screened for FAP specificity. Single chain variable fragments (scFv) from clones B1 and C11 were cloned into the IgG1 heavy chain framework in tandem with a positive control construct and a derivative EC11 immunocytokine. ScFv for FAP specificity was undertaken. PCA cell lines (PC3-N3, DU145) were engineered to express PSMA, firefly luciferase and the tTomato reporter genes. Embryonic fibroblasts (MRCS), which naturally express FAP, were transduced with the far-red mNeptune reporter gene. Co-culture ratios for CAR-MRCS were established in vitro and in vivo for the functional testing of anti-FAP-IL4 efficacy.

P.B1.05.16 Effect of N-deglycosylation on the immunogenic and antitumor properties of hemocyanins in mammals
M. Salazar1, J. M. Jiménez1, J. Villar1, M. Riveral1, M. Baeza2, A. Manubens1, M. I. Becker1; 1Fundación Ciencia y Tecnología para el Desarrollo (FUCITED), Santiago, Chile, 2Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile, 3Biosonda S.A, Santiago, Chile.

Mollusk hemocyanins from Concholepas concholepas (CH), Fissurella latimarginata (FLH) and Megathura crenulata (KLH) are glycoproteins widely used as carriers, adjuvants, and non-specific immunostimulants in cancer because they bias towards Th1 immunity. Hemocyanins are oligomeric glycoproteins (4-8 MDa), with complex disaccharide structures and heterogeneous glycosylations, mainly mannose-rich N-glycans. We have demonstrated that murine antigen presenting cells incorporate hemocyanins through mannose-recognition C-type lectin receptors (CLRs), such as Mannose Receptor (MR) and Dectin-2. However, the role of N-glycans on the immunologic properties of these proteins has not been comprehensively assessed. We hypothesized that enzymatic N-deglycosylation of hemocyanins decreases their immunogenic and antitumor effects in mammals. Hemocyanins were enzymatically and chemically deglycosylated, by treatment with peptide:N-glycosidase F and sodium periodate, respectively. Biochemical analyses showed substantial reductions in N-deglycosylated hemocyanins, and the presence of high mannose glycans. ELISA analysis showed a decreased binding of deglycosylated hemocyanins to chimeric receptors MR-Fc and Dectin-2-Fc. The humoral and antitumor responses were explored in the B16F10 melanoma melanoma model, in which mice were primed with native and N-deglycosylated hemocyanins. After 14 days, mice were challenged with melanoma cells and underwent intralesional immunotherapy. Tumor volume showed that in mice treated with N-deglycosylated KLH, compared with native KLH, while no differences were observed with N-deglycosylated CH and FLH. The specific serum antibody titer, measured by ELISA, showed a decreased humoral response in groups immunized with N-deglycosylated hemocyanins. These results suggest that N-glycosylations of hemocyanins play a role in their structure, immunogenicity, and would contribute to their antitumor potential. Funding: FONDECYT 1151337 and FONDECYT-INICEM 140151.

P.B1.05.17 Characterization of MSLN CAR T-cells in an ovarian cancer model
E. Schourop1, I. Magalhaes1, E. J. Seroff2, Y. Zhao1, M. Hasson1, J. Mattsson2; 1Karolinska Institute - Department of Oncology-Pathology, Stockholm, Sweden, 2Karolinska Institute - Department of Laboratory Medicine, Stockholm, Sweden.

Chimeric antigen receptor (CAR) T-cells are engineered to target surface antigen with the specificity of a monoclonal antibody combined with full activation of T cell effector functions. Mesothelin (MSLN) is an attractive target for CAR T-cell immunotherapy in ovarian cancer, with 60-65% of the ovarian tumors being MSLN+. Here we compare two 2nd generation MSLN directed CAR constructs containing different co-signaling domains (MSLN-CD28 and MSLN-4-1BB) and evaluate if MSLN CAR T-cells can effectively attack ovarian cancer in vitro and in vivo using an orthotopic mouse model. RD114 packaging cell lines were used for the production of γ-retroviral vectors encoding for MSLN-CD28-CAR, MSLN-4-1BB-CAR, MSLN-4-1BB-CAR, and GEP-Luciferase. Healthy donor (HV) and ovarian cancer (OC) T-cells were transduced with MSLN-CD28 and MSLN-4-1BB γ-retroviral vectors to generate MSLN CAR T-cells. The spontaneous ovarian cancer OC, Octet-3, was used as target following transduction with MSLN and GEP-Luciferase γ-retroviral vectors.
Successful generation of MSLN-CD28s and MSLN-4-1BBz CAR T-cells from HD PBMCs was established. The newly generated MSLN-CD28z and MSLN-4-1BBz CAR T-cells showed killing capacity in vitro. Stable transduction of MSLN and GFP-Luciferase vector constructs was achieved. The techniques required for MSLN CAR T-cell generation were established, as were the techniques for ovarian cancer cell transduction with MSLN and GFP-Luciferase. The latter will allow for monitoring of tumor engraftment and killing by MSLN CAR T-cells in vivo in the future orthotopic mouse model.

P.B1.05.18
Dual-specific T cells and an indirect vaccine eradicate large solid tumors
C. Y. Slaney\textsuperscript{1}, B. von Schiedt\textsuperscript{2}, A. S. Unsworth\textsuperscript{1}, P. J. Westwood\textsuperscript{1}, A. J. Davenport\textsuperscript{1}, A. Ali\textsuperscript{3}, S. Maridona\textsuperscript{1}, P. A. Beavis\textsuperscript{1}, D. S. Chuter\textsuperscript{1}, S. A. Rosenberg\textsuperscript{1}, N. P. Restifo\textsuperscript{1}, P. Neeson\textsuperscript{1}, K. F. Darcy\textsuperscript{2}, M. H. Kershaw\textsuperscript{2,3}, J. J. Gruvberger-Schill\textsuperscript{2,3}, J. D. Easton\textsuperscript{2,3}, P. J. Zelenetz\textsuperscript{1}, J. A. pikarski\textsuperscript{1}, \textsuperscript{1}John Theiler Institute, \textsuperscript{2}Sir Peter MacCallum Department of Oncology, \textsuperscript{3}Peter MacCallum Cancer Centre, Melbourne, Australia, \textsuperscript{4}Medical Oncology, \textsuperscript{5}Center for Cancer Research, \textsuperscript{6}National Cancer Institute, \textsuperscript{7}National Institute of Health, Bethesda, United States. While immunotherapy can eliminate substantial burdens of some leukemias, the ultimate challenge remains the eradication of large solid tumors and metastases for most cancers. Here we generate dual-specific T cells expressing a chimeric antigen receptor (CAR) specific for Her2 and a TCR specific for the melanocyt protein (gp100). Injection of T cells, together with a vaccine that contains a recombinant vaccinia virus expressing gp100, induced durable complete remission of a variety of Her2+ tumors and established metastases, some in excess of 150 mm\textsuperscript{3}, in immunocompetent mice expressing Her2 in normal tissues. Tumor destruction occurred rapidly over seven days and was associated with an extensive infiltrate of T cells. Mice that had rejected tumors were resistant to rechallenge with the same Her2+ tumor cells, indicating the formation of immune memory. Furthermore, we have established methods to transduce dual-specific T cells from human peripheral blood with both a TCR specific for gp100 and a CAR for Her2. From as little as 1 ml of human buffy coat, we could generate sufficient numbers of cells for a course of treatment for a patient. The stimulation of gp100 through TCR enhanced the human dual-specific CAR T cell proliferation, secretion of IFN-\gamma and killing of Her2+ human cancer cells in vitro. These characteristics were identified to be important for eradicating tumors in the mouse models. Taken together, our data provide valuable information for the development of CAR T cell therapies for patients with solid cancers and evidence for pursuing a phase I clinical trial.

P.B1.05.19
Combination therapy of CAR-NK cells and anti-PD-1 antibody displays potent efficacy against late-stage Glioblastoma and induces protective antitumor immunity
F. Strassheimer\textsuperscript{1}, C. Zhang\textsuperscript{1}, C. Mildenberger\textsuperscript{1}, R. N. Harter\textsuperscript{2}, T. Tonini\textsuperscript{1}, J. P. Steinbach\textsuperscript{1}, W. S. Weis\textsuperscript{1}, M. C. Burger\textsuperscript{1,2}.
\textsuperscript{1}Institute of Neurooncology, Goethe University, Frankfurt am Main, Germany, \textsuperscript{2}Medical Oncology, \textsuperscript{3}Radiology Institute, \textsuperscript{4}University Hospital of Frankfurt, \textsuperscript{5}Medical Oncology, \textsuperscript{6}University Hospital of Heidelberg, Germany, \textsuperscript{4}Institute for Transfusion Medicine, German Red Cross Blood Donor Service North-East and Medical Faculty Carl Gustav Carus, TU Dresden, Dresden, Germany.

Introduction: Checkpoint inhibitors as well as adoptive cell therapy hold great promise for cancer treatment and promising treatment responses have already been demonstrated in different cancer indications. Glioblastoma (GBM) is the most common and aggressive primary brain tumor. Standard therapy prolongs life expectancy only by months. Analysis of the GBM tumor microenvironment (TME) indicates elevated suppressive leukocyte infiltration. While the surrounding brain is HER2-negative, GBM tumors are frequently HER2-positive, suggesting HER2 as a promising target for adoptive immunotherapy. Indeed, previous results show efficacy of CAR-NK cells (NK-92/5.28.z) targeted to HER2 in mouse glioma models at early stages of tumor development.

Materials and Methods: The murine glioma cell line GL261 was transfected with HER2. Tumor cells were implanted subcutaneously into C57BL/6 mice and treated either with HER2-specific NK-92/5.28.z parental NK-92 cells, or in combination with anti-PD-1. Effects on tumor growth and survival were determined, and lymphocyte infiltration and immunosuppressive TME were characterized by flow cytometry.

Results: Combined treatment with NK-92/5.28.z cells and anti-PD-1 resulted in tumor regression and long-term survival of late-stage tumor bearing mice. Analysis of TME showed enhanced cytotoxic lymphocyte infiltration after treatment.

Conclusion: These data demonstrate enhanced efficacy of a combination of NK-92/5.28.z cells with checkpoint inhibitors in advanced tumors. Checkpoint inhibition possibly induces a cytotoxic rather than immunosuppressive TME, leading to a primed immune system. Thus, combination therapy may be a promising treatment goal for a clinical phase I/II study.

P.B1.05.20
Establishment and Application of a Panel of PBMC Humanized Mouse Tumor Models in Immuno-Oncology and Targeted Cancer Immunotherapy
L. Zhang\textsuperscript{1}, Y. Jin\textsuperscript{2}, H. Wu\textsuperscript{1}, F. Chen\textsuperscript{1}, L. Zhao\textsuperscript{1}, X. An\textsuperscript{1}, W. Tan\textsuperscript{1}, X. Fu\textsuperscript{1}, M. Qiao\textsuperscript{2}, Q. Shi\textsuperscript{1}, W. Yang\textsuperscript{1}
\textsuperscript{1}Crown Bioscience, San Diego, United States, \textsuperscript{2}Taihang Blood Center, Taihang, China.

Monoclonal antibodies and checkpoint blocking approaches have achieved remarkable clinical success in cancer immunotherapy. Alongside the success of anti-PD-1 and anti-PD-L1 antibodies (such as Keytruda\textsuperscript{1} and Tecentriq\textsuperscript{2}), two bispecific antibodies, catumaxomab and blinatumomab have been approved to treat cancer patients, and many more bispecific antibodies are currently in preclinical or clinical development. To meet the increasing market needs for fast, reliable, and cost effective mouse tumor model systems, we have developed a panel of PBMC humanized tumor models - the MiXeno\textsuperscript{3} platform. MiXeno models can be used for a broad spectrum of applications in I/O drug discovery, including targeted cancer immunotherapy. To validate MiXeno models for targeted cancer immunotherapy, gene expression and mutation status was profiled across the Crownbio collection of over 200 xenograft models. Specific xenograft models were selected based on their tumor antigen or gene expression levels. Models which overexpressed a variety of tumor antigens (e.g. EGFR, CD47, Braf, PD-L1, etc.) were used to develop specific MiXeno tumor models via inoculation into PBMC-humanized immunocompromised mice. Reconstitution of human immune components with human PBMCs in these tumor-bearing mice provides a useful tool to evaluate targeted immunotherapeutics including bispecific T cell engagers. Via versus host disease (GVHD) in these models can be managed by optimizing immune cell reconstitution and tumor cell engrafment. Several models from the resulting MiXeno platform have been validated using standard of care immuno-oncology drugs and characterized by immunophenotyping. Further studies are needed to expand the model collection and to extend platform applications in the I/O space.

P.B1.06.01
Tumor vaccination principles and Immunotherapy - Part 6
P.B1.06.01
Influence of ionizing radiation on bispecific antibody-redirected T cells
C. Arndt\textsuperscript{1}, D. Lindner\textsuperscript{1}, S. Koristka\textsuperscript{1}, A. Feldmann\textsuperscript{1}, N. Berndt\textsuperscript{1}, R. Bergmann\textsuperscript{1}, S. Albert\textsuperscript{1}, A. Ehninger\textsuperscript{1}, G. Ehninger\textsuperscript{1,2}, J. Steinbach\textsuperscript{1,2}, M. Bachmann\textsuperscript{1,2}
\textsuperscript{1}Helmholtz-Zentrum Berlin, Research Center for Radiation Chemistry, BER, Germany, \textsuperscript{2}University of Heidelberg, Germany.

Introduction: ionizing radiation on bispecific antibody-redirected T cells

Materials and Methods: By using a CD3-PSA bSAb, the effect of radiation on redirected T cells was examined on the example of prostate cancer. Therefore, T cells exposed to doses of 2-50 Gy were cultured with PC3-PSA cells in the presence or absence of the bSAb. Afterwards T cell proliferation, cytokine release and tumor cell lysis were analyzed.

Results: The CD3-PSA bSAb engaged γ-irradiated T cells as good as unexposed T cells resulting in an efficient tumor cell lysis. However, high doses (30-50 Gy) led to a slight decrease in anti-tumor cytotoxicity. Secretion of TNF, IFN-γ and IL-2 was enhanced after exposure of T cells to 2-20 Gy in a bSAb-dependent manner, while proliferation and S-phase survival was already impaired at doses 24 Gy.

Conclusion: γ-irradiated T cells still exert a high anti-tumor reactivity upon bSAb-mediated cross-linkage. Thus, combination with radiotherapy is a feasible and promising approach. As ionizing radiation also promotes lymphocyte infiltration via indirect mechanisms, local reduction of T cell numbers due to radiation-induced cell cycle block might be compensated.
Autophagosomes may have the potential to evoke an anti-melanoma cytotoxic T cell response. Experiments in DC-SIGN-transfectants corroborated the role of DC-SIGN as an autophagosome targeting receptor. Furthermore, DCs incubated with autophagosomes upregulated Amsterd, Amsterdam, Netherlands.

1 T. T. H. Eisden

A9E8 was rapidly internalized (t1/2) using phage display. A9E8 detected cell-surface expression of Siglec-15 protein on leukemic cell lines of the myeloid lineage as well as on donor blasts from AML patients. Siglec-15 is a rapidly internalized cell-surface antigen expressed by acute myeloid leukemia cells.

1 Laboratory of Macromolecular Therapeutics (MMCT), Department of Pharmaceutical Chemistry, Centre of Pharmaceutical Sciences, University of Vienna, Vienna, Austria;
2 Department of Medical Oncology, VU University Medical Center, Amsterdam, Netherlands;
3 Institute of Medical Sciences, Aberdeen, United Kingdom;
4 Bioengineering Institute, University of Auckland, Auckland, New Zealand;
5 1AMM Therapeutics, Amsterdam, Netherlands, 1AMM Therapeutics, Amsterdam, Netherlands, 1AMM Therapeutics, Amsterdam, Netherlands.

In summary, we could demonstrate that the tumor-restricted secretion of the high affinity CD47 binder SIRPα-Fc is a potential gene therapy approach to prevent side effects in non-tumor cells. Our approach of cancer targeted immunogene therapy circumvents these side effects by triggering the expression of CD47 blocking proteins within tumor cells. Our monoclonal antibody, A9E8, specific for AT1413- bTCE was tested in vivo, both in two mouse models, one with human PBMC, the other engrafted with a human immune system (HIS) at birth. Results: In vitro, AT1413 bTCE potently induced T-cell mediated lysis of different CD43s-expressing AML cell lines, primary AML blasts and melanoma cells. Endothelial cells with detectable, but low binding of AT1413 remained unaffected. T-cell activation and proliferation were observed only in the presence of target-expressing cells. In vivo testing of AT1413 bTCE (2 mg/kg, biweekly) revealed potent AML tumor growth inhibition of 89-99 % compared with a control bTCE. In the HIS-model, normal human hematopoietic cells remained present in AT1413 bTCE treated mice. Conclusion: Our results suggest that AT1413 bTCE, which recruits T cells to CD43s-expressing tumor cells, has therapeutic potential.

Tumor eradication through CD47 blockage and immune response induction using cancer targeted immunogene therapy

M. Billerhart, M. Schönhofer, S. Eckmann, A. Kassam, W. Polzer, M. Anton, J. Maier, A. Taschner, H. Sami, M. Ogris

The overexpression of CD47 correlates with dismal prognosis in a broad range of cancers, since the interaction of CD47 with its ligand SIRPα (on macrophages and dendritic cells) prevents cancer cell eradication. Antibodies have been evaluated in advanced clinical trials for both blocking CD47 and triggering of antibody directed cytotoxicity. Unfortunately, their systemic application causes side effects like anemia, as CD47 is also abundantly expressed on non-malignant cells including erythrocytes. Our approach of cancer targeted immunogene therapy circumvents these side effects by triggering the expression of CD47 blocking proteins within tumor cells. We have cloned a plasmid vector encoding for the secreted fusion protein SIRPα-Fc consisting of the high affinity CD47 binding protein CV1 fused to a human IgG1 Fc part. A potent bystander effect was demonstrated when transferring SIRPα-Fc containing supernatant from transfected cells to CD47 positive recipient tumor cells. SIRPα-Fc transfected and luciferase-marked human MDA-MB-231 triple negative breast cancer cells were implanted orthotopically into SCID mice and tumor growth rate was demonstrated when transferring SIRPα-Fc containing supernatant from transfected cells to CD47 positive recipient tumor cells. In two mouse models, one co-injected with human PBMC, the other engrafted with a human immune system (HIS) at birth, the SLI tumor cell signal of SIRPα-Fc transfected cells strongly decreased when compared to controls. We have cloned a plasmid vector encoding for the secreted fusion protein SIRPα-Fc consisting of the high affinity CD47 binding protein CV1 fused to a human IgG1 Fc part. A potent bystander effect was demonstrated when transferring SIRPα-Fc containing supernatant from transfected cells to CD47 positive recipient tumor cells.

We have cloned a plasmid vector encoding for the secreted fusion protein SIRPα-Fc consisting of the high affinity CD47 binding protein CV1 fused to a human IgG1 Fc part. After systemic application causes side effects like anemia, as CD47 is also abundantly expressed on non-malignant cells including erythrocytes. Our approach of cancer targeted immunogene therapy circumvents these side effects by triggering the expression of CD47 blocking proteins within tumor cells.

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Redirection of switchable UniCAR T cells against radioresistant cancer cells


Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Institute of Radiopharmaceutical Cancer Research, Dresden, Germany; German Cancer Consortium (DKTK), partner site Dresden; and German Cancer Research Center (DKFZ), Heidelberg, Germany, University CancerCenter (UCC) Carl Gustav Carus TU Dresden, Tumor Immunology, Dresden, Germany; National Cancer Institute (NCI), U.S. National Institutes of Health, Bethesda, MD, USA, Medical Clinic and Polio Clinic I, University Hospital Carl Gustav Carus Dresden, Dresden, Germany; Oncoray-National Center for Radiation Research in Oncology, Medical Faculty and University Hospital Carl Gustav Carus, TU Dresden, Germany, Dresden, Germany.

Introduction: Radiation is a common therapy for solid tumors. Unfortunately there is a high risk for the outgrowth of radioresistant tumor cells against which only limited treatment options exist. We challenged the idea whether or not a chimeric antigen receptor (CAR) engineered T lymphocytes could be used as an adjuvant immunotherapy in combination with radiotherapy. Recently, we have established universal CARs (UniCARs) to redirect a short peptide epitope (ES99) which does not exist on the surface of living cells. UniCAR T cells are exclusively redirected to tumor cells in the presence of a target module (TM) that exhibits the ES99 epitope and binds to a tumor associated antigen (TAA) on the tumor cell surface.

Materials and Methods: We used different radioresistant sublines of the head and neck cancer cell line Ca9-22. Gene expression data for certain TAAs were confirmed by flow cytometry. TM and UniCAR T cells were isolated from the variable domains of monoclonal antibodies, cloned in lentiviral vectors and purified from supernatants of permanently TM producing 313 cells. UniCAR T cells were generated by lentiviral transduction. Results: Radioresistant Ca9-22 cell lines expressed PSMA, EGFR and CD19. UniCAR TMs were created against these TAAs. Armed with these TMs UniCAR T cells efficiently killed radioresistant Ca9-22 cells in vitro and in vivo.

Conclusions: Radioresistant tumor cells can efficiently be killed by redirecting UniCAR T cells against PSMA, CD19 and EGFR and thus resistance to radiotherapy can be overcome by immunotherapy based on the UniCAR technology to these targets.

Do IgG1 or IgG4 subclasses differently affect ADCC and ADCP against EGFR+ tumor cells?

S. A. F. Jensen, G. Jordakieva, M. Bergmann, J. Laengle, S. N. Karagiannis, E. Jensen-Jarolim, R. Bianchini

Comparative Medicine, The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria, Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectology and Immunology, Medical University of Vienna, Vienna, Austria, Department of Physical Medicine, Rehabilitation and Occupational Medicine, Vienna, Austria, Division of General Surgery, Department of Surgery, Comprehensive Cancer Center Vienna, Medical University of Vienna, Vienna, Austria, St. John’s Institute of Dermatology, School of Basic & Medical Biosciences, King’s College London & NIHR Biomedical Research Centre at Guy’s and St Thomas’ Hospital and King’s College London, Guy’s Hospital, King’s College London, London, United Kingdom, Breast Cancer Now Research Unit, School of Cancer & Pharmacological Sciences, King’s College London, Guy’s Cancer Centre, London, United Kingdom.

Background: Monoclonal anti-tumor antibody treatments could be considered as one of the most successful therapeutic strategies. Their major mechanism of action is the induction of a cytotoxic immune response against tumor cells either via direct antibody-dependent cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP). Our previous in vitro studies showed that IgG4 can drive M2a macrophages to an immunoregulatory phenotype. In the light of these data we hypothesized that IgG1 and IgG4 isotypes may differentially polarize tumor-associated macrophages (TAMs), thereby impacting the quality of ADCC and ADCP against tumor cells. Methods: In vitro, monocyte-derived macrophages (MDMs) or monocyte differentiated cell line (U937) were treated with M-CSF and IL-4/IL-13 (M2a) to induce a M2-like polarization. A431 and Caco-2 tumor cell lines were used to test the anti-EGFR lgG1 or lgG4 mediated ADCC and ADCP with polarized U937 or MDM as effector cells. The analyses were performed by FACs and microscope immunofluorescence. Results: Our preliminary data demonstrate that treatment with IgG1 or IgG4 anti-EGFR influenced ADCC and ADCP activities by macrophages, resulting in a modulation of the killing effect of high EGFR expressing A431 cells compared to low EGFR expression Caco-2. Conclusion: Our results indicate that the specific use of IgG1 or IgG4 anti-EGFR antibody treatments may critically influence treatment outcome by modulating ADCC and ADCP mediated by macrophage response. We propose that the understanding of interaction of specific anti-tumor IgG subclasses with Fc-gamma receptors expressed by TAM will help to better predict the outcome of anti-cancer immunotherapies. Supported in part by Austrian Science Fund FWF projects SFB F0660-B28 to EJ.

MISTRG: improved human immune system model mouse to test transplantable T cell therapy against solid tumors


Department of Physical Medicine, Rehabilitation and Occupational Medicine, Vienna, Austria, Department of Medical Clinic and Policlinic I, University Hospital Carl Gustav Carus Dresden, Dresden, Germany, Oncoray-National Center for Radiation Research in Oncology, Medical Faculty and University Hospital Carl Gustav Carus, TU Dresden, Germany, Dresden, Germany.

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MISTRG: improved human immune system model mouse to test transplantable T cell therapy against solid tumors

P.B1.06.11

Redirection of switchable UniCAR T cells against radioresistant cancer cells


Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Institute of Radiopharmaceutical Cancer Research, Dresden, Germany; German Cancer Consortium (DKTK), partner site Dresden; and German Cancer Research Center (DKFZ), Heidelberg, Germany, University CancerCenter (UCC) Carl Gustav Carus TU Dresden, Tumor Immunology, Dresden, Germany; National Cancer Institute (NCI), U.S. National Institutes of Health, Bethesda, MD, USA, Medical Clinic and Polio Clinic I, University Hospital Carl Gustav Carus Dresden, Dresden, Germany; Oncoray-National Center for Radiation Research in Oncology, Medical Faculty and University Hospital Carl Gustav Carus, TU Dresden, Germany, Dresden, Germany.

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Conclusions: Radioresistant tumor cells can efficiently be killed by redirecting UniCAR T cells against PSMA, CD19 and EGFR and thus resistance to radiotherapy can be overcome by immunotherapy based on the UniCAR technology to these targets.
POSTER PRESENTATIONS

This model therefore more reliably mimics human tumors than traditional models using NSG mice. We will use this new model to engraft patient-derived non-small cell lung cancer (NSCLC) tumor cells and subsequently test ex vivo expanded TILs in an autologous setup. To do so, we {} platform is isolated directly from patient biopsies and expanded ex vivo as organoid culture, cell line culture, or in-vivo in a xenograft model. We will use this model to study parameters that determine the outcome of TIL therapy and to develop and test different TIL modalities.

P.B1.06.12
Minimal residual disease monitoring in course of immunotherapy of acute lymphoblastic leukemia

A. V. Komkov1, A. M. Miroshnichenko2, G. A. Nogovoy3, Y. B. Lebedev4, M. A. Masch2, I. Z. Mamedov5

1Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russian Federation, 2Dmitry Rogachev National Medical Research Center of Phsyiological Chemistry, Moscow, Russian Federation, 3National Research Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation, 4Academic Medical Center (AMC), Amsterdam, Netherlands

Anti-Cd19 immunotherapy based on CAR-T and bispecific antibodies significantly increased the efficiency of refractory leukemia treatment. However, it also brought a new challenge such as increasing frequency of Cd19-negative relapses including lineage differentiation switching. Minimal residual disease (MRD) diagnostics is the tool which allows to reveal the risk of relapse by leukemic cells concentration measurement right after therapy. The most common method for MRD diagnostics is flow cytometry which, however, has significant limitations due to target surface markers loss during immunotherapy. Here we present marker expression independent method for MRD monitoring based on high-throughput (HTS) of clonal rearrangements of T- and B-cell receptors loci. Leukemia-specific rearrangements were identified by analysis of their frequencies in sequenced amplicons obtained in multiplex PCRs with primers for vast majority of rearranged TCR and BCR loci. MRD monitoring was performed by HTS-based detection of previously identified rearrangements in samples after therapy and subsequent quantitative analysis using statistical principal of digital PCR.

The developed method was tested on cohort of 20 patients received standard chemotherapy. Over 100 clonal rearrangements were detected in initial samples in total. All identified rearrangements were used for subsequent MRD monitoring. Obtained MRD results were highly concordant with ones detected by flow cytometry. The pilot MRD monitoring by developed method after immunotherapy was performed for four patients. All these cases were MRD negative. Being highly accurate and sensitive, HTS of rearranged TCR/BCR loci is most promising alternative of flow cytometry for MRD diagnostics in leukemia immunotherapy. Fsfs: Rsg gr 17p5-1013, N18-14-0264.

P.B1.06.13
Development of a GMP-compliant two-step maturation process for the generation of plasmacytoid dendritic cells as anti-tumor vaccine

K. Kuz1, N. Smir1, T. Fecher1, E. Dimitriou1, T. Trzask1, A. Felsen1, P. Reinhardt1, P. Schuler1, J. Münch1, T. Hoffmann2, H. Schrezenmeier3, D. Fabricius1, B. Jahrsdoerfer1

1Department of Transfusion Medicine, Ulm, Germany, 2Institute for Molecular Virology, Ulm, Germany, 3Department of Oncology and Immunology, Ulm, German, 4Department of Otolaryngology, Ulm, Germany, 5Department of Pediatrics, Ulm, Germany

Allogeneic plasmacytoid dendritic cells (pDC) from partly HLA-matched healthy donors may represent a promising alternative to conventional DC as anti-tumor vaccine. Recently, GMP-compliant isolation of pDC precursors from peripheral blood became possible, so that their clinical application appears within reach. In our current study, we tested a GMP-compliant positive selection kit to isolate BDCA-4+ pDC from leukaemia products collected from 26 unstimulated healthy donors. After isolation, pDC precursors can be activated and matured within 24-48 hours. On average, we retrieved 4.4 x 10^6 pDC from 2 x 10^10 total PBMC. Purity was 95.9%, viability 94.5%. Extrapolated to the size of a full leukaemia product, 10^9 viable pDC with K562 lysis, followed by TLR-stimulation, we tested the capacity of pDC to generate cytokytic T cells via CSFE staining, intracellular IFNγ staining, and a Europium-based cytokotoxicity assay. We observed that maturation of pDC consists of a biphasic activation process. During phase 1, immature pDC express high levels of protes and few costimulatory molecules. Conoscope microscopy demonstrated that only in this phase pDC can take up antigens from K562 cell lysates. In phase 2, antigen-loaded pDC matured by TLR ligands downmodulated proteases, but upregulated costimulatory molecules and MHCII/peptide complexes. Moreover, induced pDC induce expression and proliferation of allogeneic CD8+ cytotoxic T cells. Moreover, after expansion in the presence of K562 lysis-pulsed pDC, cytotoxic T cells were able to kill my study cells. We used this study to confirm that GMP-compliant generation of pDC as allogeneic anti-tumor vaccine is feasible.

P.B1.06.14
Evaluation of TRAIL1 as a suitable receptor for intracellular drug delivery via an antibody drug conjugate.

A. Laroche1, R. Melhem1, J. Martin1, J. Joubert2, J. Lobouret1, A. Pellegrin1, M. Poul2

1RCMT - U1194, Montpellier, France, 2GICC CNRS UMR. 7292, Tours, France.

Transferrin receptor 1 (TRAIL1) is a cell surface receptor expressed on various cancers. TRAIL1 provides iron to cells for their metabolic activity by mediating the internalization of iron loaded transferrin (holo-Tf). Our lab has recently generated an internalizing anti-TRAIL1 antibody (H7) that blocks holo-Tf internalization and deprives efficiently cancer cells from iron. H7 treatment led to cell death in lymphoma and leukemia cell models and to in-phase blockade in pancreatic adenocarcinoma (PDAC) cells models, in vitro. To enhance its toxic activity, H7 was now conjugated to the microtubule inhibitor monomethyl auristatin F (MMAF).

Methods: The anti-TRAIL1 H7 was conjugated to auristatin F with a non-cleavable linker to obtain an antibody drug conjugate (ADC). Negative controls anti-Cd20 rituximab was obtained the same way. The anti-Cd30 ADC brentuximab vedotin was used as a positive control for its activity on Karpas lymphoma cell line. ADC activity was measured by MTS assay.

Results: H7-MMAF was more potent at reducing Cd30 positive Karpas lymphoma cell viability in vitro than the reference anti-Cd30 ADC brentuximab vedotin. (2H7-MMAF highly reduced BaPc3 and CFPAC PDAC cell lines viability in vivo compared to the naked antibody H7. These data suggest that receptor for microtubule inhibitor intracellular delivery in cancer. H7-ADC iron deprivation intrinsic activity likely combines to microtubule inhibitor to kill cancer cells. Toxicity assessment of H7-MMAF on normal cell lines will be crucial for further development.

P.B1.06.15
CD20 and CD37 antibodies cooperatively induce killing of malignant B cells through complement-dependent cytotoxicity


1Gennmab, Utrecht, Netherlands, 2Leiden University Medical Center, Leiden, Netherlands, 3VU University Medical Center, Amsterdam, Netherlands, 4University of Virginia School of Medicine, Charlottesville, United States, 5University of Rochester Medical Center, New York, United States.

In recent years, B-cell malignancies have been successfully targeted with anti-CD20 monoclonal antibodies (mAbs). Another interesting B-cell target is CD37, targeting of which by therapeutic antibodies is currently undergoing clinical evaluation. While known CD20 antibodies can employ complement-dependent cytotoxicity (CDC) as an efficient effector mechanism, currently known CD37 antibodies are poor inducers of CDC. Antibody engineering and drug combination studies are promising strategies to enhance antibody mediated effector functions. For example, CDC efficacy can be improved by introducing single point mutations in the Fc domain that enhance intermolecular Fc-Fc interactions between IgG molecules after cell surface antigen binding, thereby facilitating IgG hexamer formation. In this study, we explored whether introducing such a hexamerization-enhancing mutation into anti-CD20 mAbs could provide mutation in malignant B-cells, and whether CDC could further be enhanced by combinations of CD20 and CD20 mAbs. Interestingly, introducing a hexamerization-enhancing mutation into CD20 mAbs resulted in enhanced CDC of tumor B-cells. More striking, combinations of hexamerization-enhanced CD20 and CD37 mAbs showed superior CDC ex vivo in tumor cells obtained from patients with B-cell malignancies, compared to the single agents alone. Furthermore, in depth analysis into the mechanism behind enhanced CDC activity demonstrated that, upon target binding, CD20 and CD37 antibodies co-localize on the cell surface and substantially enhanced Fcγ binding and recruitment, indicating more efficient activation of complement components. These results provide a rationale for antibody combinations to enhance CDC, and provide new mechanistic insights into cooperative interactions between antibody molecules leading to highly efficient CDC activity.

P.B1.06.16
Efficacy of ex-vivo PD-1 blockade in cervical tumor-draining lymph node is related to a CD8+ T-cell subset with high levels of immune checkpoints and superior poly-functional effector functions


1Cancer Center Amsterdam, Amsterdam, Netherlands, 2Center for Gynecologic Oncology Amsterdam (CGOA), Amsterdam, Netherlands, 3Department of Medical Oncology, VUMc, Amsterdam, Netherlands, 4Academic Medical Center (AMC), Amsterdam, Netherlands, 5Department of Pathology, VUMc, Amsterdam, Netherlands, 6Netherlands Cancer Institute, Amsterdam, Netherlands.

Introduction: An important prognostic factor in cervical cancer (CxCa) is lymph node metastasis. High and interrelated rates of Tregs and PD-L1-positive macrophages in metastatic tumor-draining lymph nodes (TLDN) previously pointed to the applicability of PD-(L)1 blockade to halt metastatic spread. Here, we show the ex-vivo efficacy of PD-1 blockade in CxCa primary tumors (PT) and TLDN and relate it to the presence of a specific CD8+ T-effector cell subset.

Materials & Methods: The effect of anti-PD-1 on T-cell reactivity against the HPV65 E6 oncoprotein in TLDN (n=12) and PT (n=7) single cell suspensions was assessed after 10 days in-vitro culture by IFNγ Eliospot readout. Multicolor flowcytometric analysis of T-cells in TLDN (n=23) and PT (n=10) was also performed.
RESULTS: Consistently enhanced T-cell responses to HPV16 E6 upon PD-1 blockade were observed in all tested metastatic TDLN, but remarkably only in 1/4 HPV16+ PT. Extensive flow cytometry analysis revealed a selective and significant correlation between the ex vivo efficacy of PD-1 blockade and frequencies of CD8+ CD39+ FOXP3+ T-cells. This subset was characterized by an effector phenotype with elevated expression levels of PD-1, CTLA4, TIM3 and Lag3 checkpoints, but, rather than exhausted, was shown upon activation to express higher levels of Granzyme-B and effector cytokines as compared to its CD8+ FOXP3+ counterparts.

CONCLUSION: These data support the earlier reports of a “poised” HPV-specific T-cell repertoire in TDLN and show them to be valid targets for PD-1 blockade. Moreover, they point to CD8+ CD39+ FOXP3+ T-cells as likely therapeutic target, which may also serve as predictive biomarker.

P.B1.06.17
Modular design of a trispecific T-cell engager antibody (TriTE) for dual targeting of colorectal cancer
A. Tapia1, R. Navarro1, M. Compte1, A. Erer1, M. Zanca2, L. Alvarez-Vallina1, L. Sane2
1Molecular Immunology Unit, Hospital Puerta de Hierro, Majadahonda, Spain, 2Immunotherapy and Cell Engineering Laboratory, Department of Engineering, Aarhus University, Aarhus, Denmark.

INTRODUCTION: Bispecific T Cell Engagers (BiTE) are engineered antibody constructs composed of an anti-CD3 single-chain variable fragment (scFv) linked to an anti-tumor-associated antigen (TAA) scFv. To minimize the risk of “on-target/off-tumor” effects, we propose a dual-targeting approach with trispecific tandem antibodies recognizing simultaneously two different TAA. As a proof of concept we have designed a trispecific T-cell engager (TriTE) directed against epidermal growth factor receptor (EGFR), carcinoembryonic antigen (CEA) and CD3 for colorectal cancer immunotherapy.

MATERIALS and Methods: To generate the TriTE, an anti-EGFR and an anti-CEA single-domain VHH were cloned N-terminal and C-terminal, respectively, to an anti-CD3 scFv in the FLAG-tagged expression vector pCR3.1. Human 293T cells were transfected and conditioned medium was analyzed by Western blot, ELISA and FACS. EGFR/CD3 and CEA/CD3 binding was confirmed by FACS analysis of LITMUs (Lentivirally engineered T-cells) as well as critical path experiment (CPTE).

RESULTS: Western blot analysis demonstrated that LITE and TriTE were secreted and the migration patterns were consistent with the predicted molecular weight (43 and 57 kDa, respectively). As shown by ELISA, the TriTE was able to bind simultaneously plastic-immobilized CEA and EGFR. Moreover, it efficiently recognized the cognate antigens of the three prostate cancer cell lines in vivo, as assessed by flow cytometry.

CONCLUSIONS: To our knowledge, the TriTE is the first trispecific antibody simultaneously targeting two TAA and CD3 to redirect specifically T cells responses against colorectal cancer cells. This strategy opens the door for a new class of promising immunomodulatory antibodies in oncology.

P.B1.06.18
Targeted tumour treatment using polymer drug delivery systems
M. Sirova1, B. Rihova1, P. Chytil1, M. Tavares2, O. Luckiy1, T. Etrych1
1Inst. of Microbiology ASCR, Prague 4, Czech Republic, 2Inst. of Macromolecular Chemistry ASCR, Prague 4, Czech Republic.

Polymer-bound cytotoxic drugs represent a potential strategy of an effective tumor-targeted therapy devoid of serious side effects. Biocompatible polymer carriers ensure prolonged circulation of the drug in inactive form, its tumor-specific accumulation and controlled release in the target tissue. Water-soluble N- (2-hydroxypropyl)methacrylamide (HPMA) is one of the most promising drug carriers. Treatment of murine syngeneic tumors with HPMA-based conjugate of doxorubicin resulted in stronger infiltration of the treated tumors with immune cells, as compared with free doxorubicin. The infiltrates contained more CD8+ effector cells than the infiltrate of Dox-treated tumors. The T cells especially infiltrated PD-1+ cells, indicating a possible advantage of using checkpoint inhibition. These data conform with our previous results documenting that, upon treatment with the polymer cytotoxic drugs but not with free drugs, a complete regression of the tumors can be achieved, associated with long-lasting tumor resistance, chiefly mediated by CD8+ T cells. Indeed, the host immune system is responsible for the curative effect of the HPMA-based cytotoxic drugs. The same drug delivery system also proved good performance when delivering agents, which could reduce suppressing activity of myeloid-derived suppressor cells (MDSC), thereby potentiating the effect of polymer-bound cytotoxic drugs. Supported by projects 17-08084S and 16-28606A.

P.B1.06.19
Chondroitin sulfate proteoglycan 4 (CSPG4) is an immune target in induced drug tolerant melanoma cells (IDTCs)
K. Urano1,2, T. Kalic1, K. Karapandza1, I. Ellinger1, H. Breiteneder1, H. Schaider2, C. Haffner2
1Department of Dermatology, Karl Landsteiner University of Medicine, St. Pölten, Austria, 2Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria.

Acquired resistance to PLX4032, a selective inhibitor of mutant BRAF, remains the major obstacle in treating patients with metastatic melanoma. Induced drug-tolerant cells (IDTCs), characterized by an increase in CD271 expression, constitute a state preceding permanent drug-resistance. Chondroitin sulfat e proteoglycan 4 (CSPG4) is a multifunctional transmembrane proteoglycan, involved in sprouting, migrat ion and invasion of melanoma. We hypothesised that targeting CSPG4 on IDTCs with anti-CSPG4 antibodies may delay or prevent the development of acquired drug-resistance by simultaneous interference of multiple signaling pathways.

BRAF-mutant CSPG4 positive and negative melanoma cells were exposed to PLX4032 in order to generate IDTCs. CD271 expression was monitored as a marker of IDTCs by flow cytometry. Morphological changes were analysed by bright-field microscopy. CSPG4 expression on melanoma cells before, during and after exposure to PLX4032 was evaluated by FACS and immunofluorescence microscopy.

Exposing BRAF-mutant cells to PLX4032 led to IDTCs which were characterized by elevated CD271 expression compared to non-treated cells and by morphological changes. A lower mean fluorescence intensity of the CSPG4 signal was found in IDTCs (1594.6±28.7) compared to untreated cells (2829.5±73.9). If microscopy confirmed a decreased amount of CSPG4 on the IDTCs cell surfaces.

These results might indicate that the inhibition of mutant BRAF influences the expression of CSPG4. This provides the basis for further investigation of the use of CSPG4 as a potential immune target in IDTCs and gives the rationale for studying the role of CSPG4 in the development of permanent drug-resistance in melanoma cells. Supported by: NFB(SLS15-007) and PARRS2016, NearMiss.

P.B1.06.20
Induction of cytotoxic T-cell antitumor activity combined with chemotherapeutic approach for the treatment of colon carcinoma
K. Węgierek1, A. Szczepiak1, N. Anjer1, J. Mietrzewierska1, I. Rossowska1, T. Gospodarsz1, M. Staffa1, E. Piętasa-Plasewska1
Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wrocław, Poland.

Conjugation of methotrexate and hydroxyurea starch (MTX-HES) is an innovative solution for prolongation of MTX half-life, reduction of its side effects and enhancing of its antitumor activity. In order to improve MTX-HES therapeutic effect, we performed in vivo experiments in which the chemotherapy was supplemented with bone marrow-derived dendritic cell (BM-DC). Initially, mice with subcutaneously growing MC38 tumors received intravenously one dose of MTX or MTX-HES. On the 3rd day of chemotherapy, BM-DC were inoculated in peritumoral injections, in three consecutive weeks. Mice were sacrificed three days after the last injection of BM-DC, and antitumor activity of splenocytes was evaluated in ex vivo analyses. Chemotherapy with MTX-HES resulted in increased percentage of CD8+ T cells among splenocytes, and their tumor specific-cytotoxicity compared to control- and MTX-group. This effect was intensified when the chemotherapy was supplemented with dendritic cell-based vaccines. The treatment with MTX-HES and BM-DC increased the percentage of CD8+CD107a+ cells. Moreover, gathered data indicated that therapy using a combination of MTX-HES with BM-DC-vaccines was more effectively effective in generation of cytotoxic T cell antitumor activity. Taken together, novel MTX-HES nanocojugate is able to modulate the antitumor response more effectively than MTX. Meanwhile, its combination with cellular vaccines can improve both therapeutic effects and specific cytotoxic activity of splenocytes. The study was funded by National Science Centre, Poland (project no. 2015/19/N/026/02908).

P.B1.07.01
Targeting gangliosides-containing liposomes to human CD169+ antigen presenting cells to induce anti-tumor immune responses
A. J. Affendii1, J. Grabowska1, M. Lopez Venegas1, K. Oleksi1, A. Barbaria2, R. Mulder3, M. Ambrosini1, J. Stöckl1, G. Storm1, Y. van Kooyk1, J. M. den Haan1
1UMc, Amsterdam, Netherlands, 2Institute of Immunology, Vienna, Austria, 3Utrecht University, Utrecht, Netherlands.

Pancreatic cancer forms a major cause of cancer related deaths with a very short mean overall survival of just 6-12 months. Our previous work has already demonstrated that CD169+ macrophages can stimulate superior immune responses. Using liposomes containing CD169-binding gangliosides, we hypothesized that these liposomes could be used to target and deliver antigens to human CD169+ antigen-presenting cells (APCs). We observed that liposomes containing GM3, GD3, GM1, GD1a, GTb1, could efficiently bind and were taken up by CD169+ overexpressing THP1 cells and human monocyte-derived dendritic cells (mDCs). mDC liposome binding and uptake could be further enhanced by IFN-α-induced CD169 upregulation, and blocked using neutralizing α-CD169 antibody.
Furthermore, ganglioside-containing liposomes were taken up by human peripheral blood CD169+ monocytes, CD169+DC123+ and CD169+DC11c+ DCs, and splenic macrophages. The uptake was associated with CD169 expression. Our ongoing work will evaluate the therapeutic potential of ganglioside-containing liposomes using imaging flow cytometry. To conclude, several ganglioside-containing liposomes bind to human CD169 and could potentially be used to target CD169+ APCs. Future studies will focus on whether these liposomes can be used to induce pancreatic tumor antigen-specific T cell responses.

P.B.1.07.02

TMV vaccine induces growth of squash mal cell carcinoma of head and neck in mice

R. Bommerdiy,1 L. E. Munoz,2 C. D. Pack,3 S. Ramachandiran,3 S. J. Reddy,1 J. Kim,1 G. Chen,1 N. F. Sabat,1 D. M. Shiri,1 P. Selvaraj1

1Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, United States; 2Maelapse Therapeutics Corporation, Atlanta, United States; 3Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, United States.

Introduction: Head and neck cancer is a leading cause of cancer related deaths accounting for approximately 3% of all cancer related mortalities in the US. Currently, there is no cure for the advanced squamous cell carcinoma of the head and neck (SCCHN) thus development of efficacious therapies is urgently needed. To test whether vaccine-induced immunity inhibits tumor growth, we investigated efficacy of a tumor membrane-based vaccine immunotherapy in a murine SCC (SCC VII) model.

Materials and Methods: The SCC VII tumors grown subcutaneously in C3H/HeJ mice were harvested to generate tumor membrane vesicles (TMVs). TMVs were then protein transferred with glycolipid-anchored immunostimulatory molecules GPI-B7.1 and GPI-IL-12 to generate the TMV vaccine. Mice were vaccinated with TMV vaccine either before (prophylactically) or after SCC VII tumor cell challenge (therapeutic) and tumor growth was monitored every 3 days. Survival was then assessed using a Kaplan-Meier survival curve and significance determined using a Log-rank test for comparison analysis.

Results: SCC VII cells express MHC I, CD44, CD47 and respond to IFNγ in vitro. The TMV vaccine inhibited tumor growth and improved the survival of mice challenged with SCC VII tumor cells further.

Conclusions: These observations suggest that tumor tissue-based vaccines can be harnessed to develop an effective immunotherapy for squamous cell carcinoma of the head and neck. Funding: Supported by Head and Neck SPORE pilot funding from Emory Winship Cancer Institute.

P.B.1.07.03

DNA prime-boost peptide boost immunizations maximize circulating and resident memory CD8+ T cell responses against a melanoma-associated self-antigen

P. Caceres-Morgado,2 F. Galvez-Cancino,1 X. Diaz,1 E. Menares,1 S. Hidalgo,1 O. Chavar,1 J. Saavedra,1 A. Llader; Fundación Cienncia & Vida, Santiago (Nuñoa), Chile.

Most cancer responses can mediate long lasting protection against cancers. Emerging evidence indicates that effective anti-tumor protection requires coordinated action of both tissue-resident and circulating memory CD8+ T cells. However, clinically applicable vaccination strategies that effectively establish both types of responses against tumor-associated self-antigens remain largely unexplored and are expected to strongly protect against tumors. Here we demonstrate that intradermal vaccination using a DNA prime-peptide boost strategy induces strong CD8+ T cell effector responses against a model of melanoma-associated self-antigen GP100 in mice, as compared to DNA and peptide prime and the other immunization regimens. These responses were characterized by a high proportion of memory precursor (KLRG1-CD127+) cells expressing CCR7. At the memory phase, DNA prime-peptide boost lead to enhanced circulating and skin resident memory (Tm) CD8+ T cells. Tm cells accumulated at both vaccinated and distant sites, and displayed elevated expression of PD-1 and low expression of Lag3 and KLRG1. Interestingly, in vivo stimulation with H-2 Db-restricted GP10041-50, peptide lead to the production of high levels of IFNγ by Tm cells. Current efforts of this project seek to demonstrate that prime and boost vaccination strategies designed to maximize resident and circulating memory CD8+ T cell responses achieve potent protection against primary and disseminated melanoma.

P.B.1.07.04

CD169+ macrophages and the development of anti-cancer vaccines

D. van Dinh1,1 J. Grabowska1,1 A. J. Affandi1, M. Lopez Venegas1,1 A. Barbaria1, H. Veninga1,1 E. Borghi1, L. Hoogterp1, K. Olesek1,1 H. Kalay1, M. Ambrosini1,1 G. Storm1, Y. van Kooyk,1 J. M. M. den Haan1

1VU University Medical Center, Amsterdam, Netherlands, 2Utrecht University, Utrecht, Netherlands.

CD169+ macrophages are present in lymphoid organs at the site of antigen entrance and are essential in the activation of innate as well as adaptive immune responses. Our aim is to target tumor antigens to these CD169+ macrophages for the activation of anti-cancer immune responses and have investigated two approaches. First, using antiCD169 antibodies and second, we show that CD169+ macrophages act as antigen presenting to B cells and stimulate strong T cell responses. Furthermore, antigens targeted to CD169+ macrophages were transferred to cross-presenting dendritic cells and this led to strong cytotoxic and helper T cell responses. Both (melanoma) peptide as well as protein antigen targeting to CD169 resulted in strong primary and recall immune responses and protective immunity against melanoma outgrowth in mice. Second, we used liposomes containing CD169-binding ganglioside ligands to target antigens to CD169+ antigen presenting cells in mice and man. These liposomes specifically bound to CD169+ macrophages in mice and to B and T cell responses against antigen present in the liposome. In addition, CD169 ligand-containing liposomes bound to human monocyte-derived dendritic cells and peripheral blood CD169+ dendritic cells and will be further evaluated for their capacity to stimulate T cell responses. In conclusion, different approaches to target tumor antigens to CD169+ antigen presenting cells demonstrate a strong capacity to stimulate immune responses and should be further explored as a vaccination strategy for cancer.

P.B.1.07.05

Tumor-derived microvesicles enhance cross-ability of cancer prognosis and chemotherapy of grade and clinical grade dendritic cells

M. Donzelli1, C. De Angelis,1 F. Battiotti,1 H. Rahimi Koshkaki,1 A. J. Zizzi,1 C. Napoletano,1 C. Albano,1 I. Ruscito,1 M. Nuti,1 A. Rughetti; Sapienza University of Rome, Department of Experimental Medicine, Rome, Italy.

Introduction: Manufacturing clinical grade Dendritic Cells (DCs) represents a critical step in tumor immunization strategies. Indeed, DC performance is affected by clinical grade culture strategies. Thus, the design of immunogens enhancing antigen presentation of Clinical Grade DCs (gDCs) is mandatory. Tumor-derived microvesicles (T-MVs) trigger protective anti-tumor immune responses by delivering tumor antigen repertoire and activatory signals to DCs. T-MVs modulate DC phagosomal alkalization allowing cross-presentation of tumor glycosylated antigens such as MUC1. Here we show that phagosomal performance of gDCs is altered (low pH, reduced phagocytosis) and that T-MVs uptake counteracts phagosomal acidification of gDCs, restoring MUC1 antigen cross-processing.

Methods: gDCs generated in X-Vivo medium and standard DCs (sDCs) grown in RPMI+10%FCS were pulsed with soluble recombinant MUC1 glycoprotein (rMUC1) or T-MVs carrying MUC1. Phagosomal pH and phagocytosis were assessed by flow cytometry, “chasing” DCs with 3μm FITC/FluoProbes647 coupled microbeads. Internalization and cross-presentation of the MUC1 carried by T-MVs or rMUC1 were evaluated by immunofluorescence, employing HLA I and II compartment markers.

Results: gDCs, compared to sDCs, displayed a more acidic phagosomes and a decreased phagocytosis. gDCs also were less efficient in the internalization of soluble rMUC1 glycoprotein and internalized antigen was blocked in HLA class II compartment. Pulsing gDCs with T-MVs, an increase in antigen up-take and MUC1 translocation in HLA class I compartment were observed. Indeed, T-MVs up-take triggers an early phagosomal alkalization in gDCs allowing MUC1 cross-processing.

Conclusions: T-MVs are able to reprogram DC antigen presenting machinery and represent optimal cell-free based immunogens for clinical use.

P.B.1.07.06

Generation of multiepitope cancer vaccines based on large combinatorial libraries of survivin-derived mutant epitopes

A. N. Domínguez-Romero1, M. E. Munguia-Zamudio1, F. Martinez-Cortés1, K. Manoutcharian; Instituto de Investigaciones Biomédicas, México CDMX, Mexico.

Introduction: Immune tolerance is the main challenge in the field of cancer vaccines, therefore, mutated versions of a wild-type epitope of tumor-associated antigens represent a promising pathway for these vaccines. A novel vaccine approach was developed by our research group based on the generation of a new class of vaccine immunogens, called Variable Epitope Libraries (VELs) bearing combinatorial libraries of mutated versions of wild-type immunodominant epitopes of cytoplasmic T lymphocytes. Previously, we demonstrated statistically significant tumor growth inhibition in BALB/c mice immunized with the VELs based on a survivin mutant, immunodominant epitope. Now, we used larger regions of survivin (40-45 amino acid long) to generate the VELs as multiepitope vaccines. Materials and Methods: These VELs were expressed at high copy numbers on recombinant M13 bacteriophages as peptides linked to the cphVIII phage protein by Phage Display. A 4T1 murine breast cancer model was used. The VELs were applied in therapeutic treatment studies and administered as a single intravenous injection. Results: Our preliminary data showed that a single dose of the survivin-derived VEL vaccine significantly decreased tumor growth and did not metastasize. Also, a significant reduction of MDSC cells in the lungs and tumor infiltration by CD8 T lymphocytes were observed. Conclusions: This study provides the feasibility of the generation of VEL-based vaccine immunogens as an alternative approach for the construction of cancer vaccines. Acknowledgments: Funding provided by DGAPA-UNAM (IN205216) A. Domínguez-Romero is a recipient of a scholarship from CONACyT and Postgrado en Ciencias Biológicas, UNAM.
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P.B1.07.07
Using combinatorial peptide library to design super-agonists for improved cancer immunotherapy
S. A. E. Galloway
1G. Ghalamfarsa
1A. Rastegari
2A. Masjedi
1Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran, Islamic Republic of,
2Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of,
3Department of Biopharmaceutical Sciences, University of California, Irvine, Irvine, United States

The use of peptide vaccines for the treatment of melanoma has been a largely unsuccessful endeavour. Generally, peptides derived from tumour associated antigens (TAA) are poorly immunogenic and unable to elicit a substantial CD8 T-cell response capable of mediating effective tumour regression. Here, we demonstrate that combinatorial peptide library (CPL) screens are an important tool to design agonist peptides capable of improving the CD8 T-cell response to a commonly overexpressed TAA, melan-A. Combinatorial peptide screening of key CD8 T-cell clones, isolated from a patient who successfully cleared melanoma using TIL therapy, revealed preferred amino acid residues at each position in the cognate peptide, leading to the selection of a panel of 10 potential agonist peptides. 2D peptide arrays revealed a large percentage of peptides induced CD8- and CD4-specific responses. However, ex vivo restimulation and subsequent readout of splenocytes from anti-PD-L1 treated mice revealed a modest percentage of CD8-specific responses. Further optimization of the combinatorial peptide library is required to produce potential therapeutic peptides for the treatment of melanoma.

P.B1.07.08
Anti angiogenic Effects of CD73 specific sRNA Loaded Nanoparticles in Breast Cancer Bearing Mice
J. Grabowska
1S. A. E. Galloway
1G. Ghalamfarsa
1A. Rastegari
2A. Masjedi
1Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran, Islamic Republic of,
2Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of

Using combinatorial peptide libraries to design super-agonists for improved cancer immunotherapy
S. A. E. Galloway
1G. Ghalamfarsa
1A. Rastegari
2A. Masjedi
1Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran, Islamic Republic of,
2Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of

The effect of PCI was confirmed through induction of complex formation and surface presentation, and a subsequent 30-100-fold more efficient activation of antigen-specific CTLs compared to using the peptide alone. The effect was more pronounced when using GM3/ovalbumin-containing liposomes, which bind to CD169+ macrophages in a sialic acid dependent manner and stimulated ovalbumin-specific CD8 and CD4 T cell and B cell responses. Surprisingly, liposomes without GM3 also stimulated immune responses. As expected, CD169+ macrophages strongly and specifically bound GM3 liposomes, while macrophages from sialoadhesin knock-in mice bearing a mutation in the CD169 ligand binding pocket were incapable of binding GM3 liposomes. After intravenous administration, GM3/ovalbumin-containing liposomes specifically bound to CD169+ macrophages in the liver and spleen of GM3 knock-in mice, while GM3 knock-in mice did not show GM3 binding. Further studies will be needed to determine the fate of GM3 liposomes in vivo. In conclusion, PCI is an emerging technology route endocytosed material to the cytosol of cells, based on light-induced disruption of endosomal membranes using a photosensitizing compound. Here we investigated the potential of PCI, as a minimally invasive and well-tolerated vaccination technology to induce priming of cancer-specific CTL responses to peptide antigens.

P.B1.07.09
Evaluation of GM3-containing liposomes for antigen targeting to splenic CD169+ macrophages to induce anti-cancer immunity
J. Grabowska
1S. A. E. Galloway
1G. Ghalamfarsa
1A. Rastegari
2A. Masjedi
1Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran, Islamic Republic of,
2Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of

Using combinatorial peptide libraries to design super-agonists for improved cancer immunotherapy
S. A. E. Galloway
1G. Ghalamfarsa
1A. Rastegari
2A. Masjedi
1Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran, Islamic Republic of,
2Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of

Photochemical internalization (PCI) provides an emerging technology to route endocytosed material to the cytosol of cells, based on light-induced disruption of endosomal membranes using a photosensitizing compound. Here we investigated the potential of PCI, as a minimally invasive and well-tolerated vaccination technology to induce priming of cancer-specific CTL responses to peptide antigens. We show that PCI effectively promotes delivery of antigen peptides to the cytosol of antigen presenting cells in vitro. This resulted in a 30-fold increase in MHC class I/peptide complex formation and surface presentation, and a subsequent 30-100-fold more efficient activation of antigen-specific CTLs compared to using the peptide alone. The effect was found to be highly dependent on the dose of the PCI treatment, where optimal doses promoted maturation of immature dendritic cells, thus also providing an adjuvant effect. The effect of PCI was confirmed in vivo by the successful induction of antigen-specific CTL responses to cancer antigens in C57BL/6 mice following intradermal peptide vaccination using PCI technology. We show thus new and strong evidence that PCI technology holds great potential as a novel strategy for improving the outcome of peptide vaccines aimed at triggering cancer-specific CD8+ CTL responses.

P.B1.07.10
Discovery of immunogenic neoantigens for peptide vaccination approaches in murine colorectal cancer.
B. J. Nos
1IHB, LUMC, Leiden, Netherlands

Recent developments have shown that effectiveness of therapy with checkpoint-blocking antibodies correlates with the expansion and invigoration of neo-antigen specific T cells. Alongside, peptide-based vaccines targeting onco-viral antigens have shown to be effective inducers of T cell responses related to reduction of HPV-induced pre-malignancies. This suggests that peptide-based vaccination targeting neoantigens is a viable immunotherapeutic strategy. A major limitation for broad application of peptide-based vaccinations is the characterization of cancer-specific epitopes that is required for personalized approaches. The process of epitope identification is yet not trivial. Here we describe the process in a murine colorectal cancer model to identify immunogenic epitopes for peptide vaccination. DNA and RNA analysis revealed the expression of several thousand mutations. Prediction and ranking of MHC class I binding peptides containing mutations with NetMHC4.0 limited this number to several hundred high- and moderate-affinity epitopes. An immunogenicity test of 57 selected high-affinity peptides (based on the type of amino-acid substitution) revealed a large percentage of peptides induced CD8- and CD4-specific responses. However, ex vivo restimulation and subsequent readout of splenocytes from anti-PD-L1 treated and tumor-regressed mice, showed specific responses to a limited number of three peptides. Strong responses were observed to a novel mutated peptide sequence. Mass spectrometry could confirm the expression and presentation of this epitope, but not the other two. Our approach was successful in the characterization of immunogenic and relevant epitopes. Further research is now needed to improve effectiveness in therapeutic vaccinations.

P.B1.07.11
Discovery of immunogenic neoantigens for peptide vaccination approaches in murine colorectal cancer.
B. J. Nos
1IHB, LUMC, Leiden, Netherlands

Recent developments have shown that effectiveness of therapy with checkpoint-blocking antibodies correlates with the expansion and invigoration of neo-antigen specific T cells. Alongside, peptide-based vaccines targeting onco-viral antigens have shown to be effective inducers of T cell responses related to reduction of HPV-induced pre-malignancies. This suggests that peptide-based vaccination targeting neoantigens is a viable immunotherapeutic strategy. A major limitation for broad application of peptide-based vaccinations is the characterization of cancer-specific epitopes that is required for personalized approaches. The process of epitope identification is yet not trivial. Here we describe the process in a murine colorectal cancer model to identify immunogenic epitopes for peptide vaccination. DNA and RNA analysis revealed the expression of several thousand mutations. Prediction and ranking of MHC class I binding peptides containing mutations with NetMHC4.0 limited this number to several hundred high- and moderate-affinity epitopes. An immunogenicity test of 57 selected high-affinity peptides (based on the type of amino-acid substitution) revealed a large percentage of peptides induced CD8- and CD4-specific responses. However, ex vivo restimulation and subsequent readout of splenocytes from anti-PD-L1 treated and tumor-regressed mice, showed specific responses to a limited number of three peptides. Strong responses were observed to a novel mutated peptide sequence. Mass spectrometry could confirm the expression and presentation of this epitope, but not the other two. Our approach was successful in the characterization of immunogenic and relevant epitopes. Further research is now needed to improve effectiveness in therapeutic vaccinations.
POSTER PRESENTATIONS

P.B1.07.12
Identifying the role of microRNAs in MDS with particular focus on the contribution of immune cells

A. Kindermann1, F. Heidel2, H. Kießlenstein3, D. Quondt4
1Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Germany, 2Department of Hematology and Medical Oncology, Friedrich-Schiller University Jena, Germany.

Introduction: Myelodysplastic syndromes (MDS) are clonal hematopoietic neoplasia characterized by peripheral cytopenia with defective hematopoiesis and variable tendency to evolve into acute leukemia. MicroRNAs are known to be tight regulators of normal hematopoiesis but also to be dysregulated in various types of cancer and therefore are tumor suppressive. MicroRNAs are differentially expressed in MDS but the molecular mechanism remains elusive. Altered microRNA expression can evolve from and affect all different cell types that constitute the stem cell niche. Methods: Allogeneic tumor-T cell co-culture assays with myeloid leukemic as well as MDS cell lines could be successfully established. The use of Viromer transduction was available to overexpress miR-34a using mimics in primary human T cells and leukemic cell lines as determined by qPCR. Differential bone marrow cell sorting for early hematopoietic stem cells (CD34+, Lin neg, CD38 neg), committed stem cells as well as T cells will allow the generation of distinct microRNA profiles. Results: Overexpression of miR-34a did not alter subsequent proliferative behaviour of polyclonal activated T cells. Tumor T cell co-culture assays with altered miR-34a expression on the tumor site did not yet give conclusive results of an impact on T cell activation. The FACS sorting strategy for differential bone marrow cells could be established and high quality RNA from 100 cells could be isolated. Conclusion: MiR-34a seems not to contribute primarily to T cell-tumor communication. The role of this microRNA for myeloid progenitor survival, proliferation and differentiation will be investigated in ongoing experiments.

P.B1.07.13
Dendritic cells pulsed with tumor cells killed by high hydrostatic pressure are effective as prostate cancer immunotherapy using clinically relevant murine models

R. Milikowsky1, I. Slápek1, I. Traxova2, I. Moxerová3, J. Fučíková-0, J. Bartošilka0, R. Spisek0, M. Reinos0
1Institute of Molecular Genetics AS CR, v.v.i., Prague 4, Czech Republic, 2SOTIO, Prague 7, Czech Republic, 3 Faculty of Medicine and University Hospital Motol, Charles University, Prague 4, Czech Republic.

High hydrostatic pressure (HP) has been proved to induce immunogenic cell death of cancer cells and dendritic cell (DC)-based vaccines pulsed with HP-inactivated tumor cells have recently been shown to be a promising tool for immunotherapy. In this study, we analyzed their therapeutic efficacy in clinically relevant settings, such as chemoinmunotherapy, surgical minimal residual tumour disease and, finally, in the orthotopic transgene adenocarcinoma of the mouse prostate (TRAMP) model, which mimics what happens in human disease. In this model, high pressure inactivates tumor cells with docetaxel (DTX) chemotherapy inhibited growth of both TRAMP-C2 and TC-1 tumors. Administration of these vaccines after the surgical removal of tumors slowed down the growth of tumor recurrences. Finally, pulse-activated DCs were also useful in reducing prostate cancer growth in the TRAMP model when used alone or in the combination with docetaxel. Although we did not observe any additive or synergistic effects of chemotherapeutant, the combination of DTX and pulsed dendritic cells resulted in significantly lower tumor cells proliferation (detected by Ki67 staining) in growing tumors. Collectively, our results indicate that the DC-based vaccines pulsed with HP-inactivated tumor cells represent a suitable tool for immunotherapy, particularly with regard to the findings that poorly immunogenic TRAMP-C2 tumors were susceptible to this treatment modality. This work was supported by research grants provided by SOTIO a.s. and by Academy of Sciences of the Czech Republic (RVO 68378050).

P.B1.07.14
The in vitro melanoma tumor microenvironment conditions macrophages to an immunosuppressive M2-like phenotype, which is reversible by lipopolysaccharide and interferon-gamma

I. Milenova1, M. Lopez Gonzalez1, T. Brachtlova, R. van de Ven1, W. Dong1, W. V. van Beusechem3, R. de Gruij2
1Vrije Universiteit Medical Center/ORCA Therapeutics, Amsterdam, Netherlands, 2Vrije Universiteit Medical Center, Amsterdam, Netherlands, 3ORCA Therapeutics, Amsterdam, Netherlands.

The melanoma tumor microenvironment is conditioned to skew infiltrating monocytes to M2-like macrophages. These M2-like macrophages promote growth and invasion of tumor cells. We are exploring the use of oncolytic adenoviruses to enhance the efficacy of immunotherapy and induce favorable M1-like polarization.

In order to study macrophage polarization in the tumor microenvironment, human melanoma cell lines were co-cultured in vitro with CD14+ monocytes. Lipopolysaccharide (LPS) and interferon (IFN)-gamma were added to the co-culture to assess M1 polarization, and the effect of the oncolytic adenovirus ORCA-010, which is modified to facilitate improved oncolysis. We demonstrate that the melanoma cells strongly induce an M2-like macrophage phenotype (CD14+/CD63+ /CD80-/CD86-/PD-L1+) in vitro. Whereas addition of ORCA-010 led to upregulation of CD80, the combination of LPS and IFN-gamma proved able to negate these effects and induce an M1-like phenotype (CD14+/CD63-/CD80+/CD86+/PD-L1+). These findings demonstrate the feasibility of M1 skewing in the face of melanoma-induced immune suppression.

Next, the macrophage polarizing effects of various clinically applicable immune modulatory agents will be studied. In the B16-OVA melanoma model we are investigating the effects of the combined administration of ORCA-010, a p88-MAPK inhibitor and anti-PD-1. We have observed in vitro inhibition of p38 signaling to skew macrophage differentiation away from a melanoma-imposed immunosuppressive M2-like phenotype. We hypothesize that this will result in increased T cell infiltration and activation at the tumor site, thus increasing the efficacy of PD-1 checkpoint blockade.

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P.B1.07.15
Are you my MAIT?- Investigating the role of MAIT cells in Colorectal-Liver Metastases

R. M. Millen1, S. Rohli1, P. Beavis2, B. Thompson3, S. Banting4, J. Kelly5, N. A. Gherardin5, D. Godfrey6, X. Visvanathan6, R. G. Ramsay6
1University of Turin, Turin, Italy, 2Agilvax Inc., Albuquerque, United States, 3Peter MacCallum Cancer Centre, St. Vincent’s Hospital, Melbourne, VIC, Australia, 4Peter MacCallum Cancer Centre, Melbourne, VIC, Australia, 5The Fiona Elsey Cancer Research Institute, Ballarat, VIC, Australia, 6Peter Doherty Institute, Melbourne, VIC, Australia.

Background: Stage-IV Colorectal Cancer (CRC) involves metastasis to the liver. Cytotoxic CD8+ T-cells play a critical role in cancer; serving as a key prognostic marker for the Galon-Immunoscore. 7

Methods: Associated Invariant T-cells (MAITs) are a recently described subset of T-cells that co-express CD8, and are important in controlling bacterial infections. MAITs are highly abundant in the liver comprising up to 40% of T-cells. The role of MAITs in solid tumours is starting to emerge and it’s likely they are included with CD8+ cells when defining Tumour Infiltrating Lymphocytes (TILs). MAIT cells may be prognostic and may be a novel immune-therapeutic target.

Results: We have recruited 25 patients with liver metastases and documented MAITs in the tumour; surrounding liver tissue and PBMCs. MAIT cell frequency is decreased in the tumour compared to the surrounding tissue. However, MAITs have a high expression of PD-1 in the tumour, indicating potential regulatory role in immune checkpoint blockade (ICB). We have preliminary data demonstrating that autologous MAIT cells have effector function when co-cultured with patient-derived tumouroids in vitro.

Conclusions: MAITs are decreased in the tumour, with high PD-1 expression and may be ideal targets for ICB. These intriguing results warrant further investigation to determine their direct or indirect role of MAIT cells in tumour immunity.

P.B1.07.16
Virus like particle vaccine targeting xCT protein in preclinical breast cancer models

V. Rolhi1, E. Bolliv, L. Cont1, J. M. Christen1, J. Caldeira1, F. Perciè1, E. Cavall1
1University of Turin, Turin, Italy, 2Agilvax Inc., Albuquerque, United States.

Cancer stem cells (CSCs) are involved in the resistance mechanism to traditional radio- and chemo-therapies. They represent a reservoir for relapse and metastatic evolution. The cystine-glutamate antiporter xCT (SLC7A11) has been identified as over-expressed on the cell surface of breast CSC (BCSC) and essential for their resistance to common cytotoxic therapies. xCT expression is linked to a few normal cell types, in particular neural crest cells and melanoma. The role of xCT mRNA and protein correlate with significant reduction in distal metastases-free and overall survival. Thus, xCT could be an ideal oncoantigen for immunotherapy. We have developed an innovative vaccine based on a virus-like-particle (VLP) technology targeting the xCT protein for breast cancer treatment.

Immunization with our VLPs elicited a strong antibody response against xCT and these antibodies affected BSCC function and biology in vitro. We studied the effect of our vaccine on metastasis formation in two different settings: as a preventive model in BALB/c mice injected with Her2+ TUBO cells and as a therapeutic model in BALB/c mice injected with triple negative 4T1 cells. For both protocols, treated mice had a significant reduction in lung metastases compared to controls. These data show that our VLP vaccine can inhibit xCT activity, impact CSC biology and significantly reduce metastatic progression in preclinical models. We are studying the association of our VLPs vaccine with anti-PD-1 monoclonal antibody therapy. In the future, we would like to translate this combinational approach to the clinical settings.

Abstracts of the 5th European Symposium on Clinical Immunology - ECI 2018 - Amsterdam, The Netherlands 237
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.1.B1.07.17
Selection of tumor cells in the cultivation stage, suitable for the development of a vaccine against urothelial cancer
T. Slavynskaya1, S. Salnikova2
1Peoples Friendship University of Russia (RUDN University), Moscow, Russian Federation; 2FSBI Clinical Hospital №1 of the Presidential Affairs Management of the Russian Federation, Moscow, Russian Federation.

Introduction: The development of vaccines for urothelial carcinoma (UCV) covers many aspects necessary for its standardization. Materials and Methods: We assayed UC samples from 25 patients aged 37-82. Cultivation of UCC was performed on growth media DMEM/F12. Evaluation of viability (V) was performed every 24 h for 6 days. Tumor development was performed by means of 3 multiparametric methods: morphometric and enzymatic methods. Cancer-testicular antigens (CTA) expression was evaluated at FACScan Canto II device. Software package SPSS 23.0 for Windows was used. Results: V of UCC in a 1st day exposure at a temperature (T) 0°C was fall down on 7.1±0.4%, at +4°C on 3.3±0.4%, at +8°C on 11.8±1.5% (p≤0.05). From 2 to 6 days V was decreased on 9.0±2.6% and on 66±7.4%, respectively. V of the UCC during the mechanical disaggregation was significantly higher than 81±6% (p≤0.05) compared to the enzymatic method: 28±1.3% or 55±2.9%, depending of UC forms. In UCC cultures CTA expression was detected frequently: Mage 70%, BAGE 30%, GAGE 40%, NY 650-1 50%. At early passages quantity of UC, expressing CTA, analyzed to 75±10%. Conclusions: Thus, in MNC UC CTA expression is significantly lower, than in case of UC. Particular cultures in the course of multiple tests preserved cytogenetic profile and consistent CTA expression, which makes them promising for preparation of antitumor vaccine in case of UC. The publication has been prepared with the support of the “RUDN University Program 5-100”.

P.1.B1.07.18
Genetic mutations of tumor cells depending on the clinical forms of urothelial cancer
T. Slavynskaya1, S. Salnikova2
1Peoples Friendship University of Russia (RUDN University), Moscow, Russian Federation; 2FSBI Clinical Hospital №1 of the Presidential Affairs Management of the Russian Federation, Moscow, Russian Federation.

Introduction: The objective of the work is to perform comparative study of genetic mutations and expression of cancer-testicular antigens (CTA) in case of different forms of UC. Materials and Methods: The tumor specimens (TS) of UC were studied in 54 patients aged 37-82 with muscular invasive (MIF, TS-75%) and muscular non-invasive (MNIF, TS-25%) forms. Expression of CTA was assessed by FlowCytometry method. We used the package of statistical software SPSS 23.0 for Windows. Results: It was established, that all researched TS had cytogenetic changes: deletions of 9 chromosome (Ch) - 66.7%, absence of Y Ch (50%) and monosomy of 13 Ch (33.3%). In rare cases changes in 1,3,7 Ch, monosomy of 17 Ch, trisomy of 7 Ch were detected. In case of prolonged passageing in a part of cultures, significant increase of the number of genetic changes in a form of division of previously homogenous population into subclasses differing in ploidy (up to 56 Ch) and quantity of changed Ch is observed. Certain correlation of these changes to decrease of CTA expression (Mage, GAGE, BAGE, NY-ESO-1 p≤0.05) was established. Conclusion: Thus, in MNC UC CTA expression is significantly lower, than in case of UC. Particular cultures in the course of multiple tests preserved cytogenetic profile and consistent CTA expression, which makes them promising for preparation of antitumor vaccine in case of UC. The publication has been prepared with the support of the “RUDN University Program 5-100”.

P.1.B1.07.19
Immunisation with a synthetic vaccine consisting of a tumour-associated MUC1-glycopeptide conjugated to Tetanus Toxoid significantly reduced breast tumour growth
N. Stergiou1, N. Gaidaik2, A. Heimes3, S. Dietzen4, P. Besenius2, J. Jäkel5, W. Brenner5, M. Schmidt5, H. Kunz6, E. Schmitt1
1Institute of Immunology, Mainz, Germany; 2Institute of Organic Chemistry, Mainz, Germany; 3Department of Obstetrics and Women's Health, Mainz, Germany; 4Institute of Pathology, Mainz, Germany.

Immunoassay against strongly expressed tumour-associated endogenous antigens is considered to be an attractive strategy for the induction of a curative immune response concomitant with a long lasting immunological memory. The mucin MUC1 is a very promising tumour antigen, as its tumour-associated form significantly differs from the glycoprotein form expressed on healthy cells. Due to aberrant glycosylation in tumour cells, the specific peptide epitopes in its backbone are accessible and can be bound by antibodies induced by vaccination. Breast cancer patients develop per se only low levels of T cells and antibodies recognizing tumour-associated MUC1 and clinical trials with tumour-associated MUC1 gained unsatisfactory therapeutic effects indicating urgent need to improve humoral immunity against this tumour entity. Herein, we demonstrate that preventive vaccination against tumour-associated human MUC1 results in strong specific humoral immune responses, in a marked slowdown of tumour progression and in an increased survival of breast-tumour-bearing mice. For preventive vaccination, we used a synthetic vaccine containing a specific tumour-associated glycopeptide structure of human MUC1 coupled to Tetanus Toxoid. The glycopeptide consists of a 22mer huMUC1 peptide with two immune dominant regions (PDTTR and GSTA), glycosylated with the sialylated alpha/2,3-linked oligosaccharides. The antigen was conjugated to the C-terminal end of the homogeneous Tetanus Toxoid (TNT) by a synthetic linker. The vaccine was injected subcutaneously into female BALB/c mice in a dose of 10 mg per animal and in a total amount of 50 mg per animal. Breast cancer model. The publication has been prepared with the support of the “RUDN University Program 5-100”.

P.1.B1.07.20
Tumor vaccination: chitosan nanoparticles to improve the antigen uptake by dendritic cells for an enhanced tumor-directed immune response
E. Winter1, O. Helm2, M. Lettau3, J. Heidland4, R. Scherloff3, F. Walter3, S. Sebens1
1Institute of Experimental Cancer Research, Christian-Albrechts-University (CAU) Kiel and University Hospital of Schleswig-Holstein (UKSH) Campus Kiel, Kiel, Germany; 2Institute of Immunology, Christian-Albrechts-University (CAU) Kiel and University Hospital of Schleswig-Holstein (UKSH) Campus Kiel, Kiel, Germany; 3Department of Pharmaceutics and Biopharmaceutics, Christian-Albrechts-University (CAU) Kiel, Kiel, Germany.

Dendritic cells (DCs) play a key role in the initiation of an anti-tumor immune response. They present endocytosed tumor antigens via cross-presentation on major histocompatibility complex class I molecules thereby activating CD8+ cytotoxic T lymphocytes.

However, convincing results from studies on solid tumors are still missing. One reason might be an inefficient antigen uptake by DCs. The aim of this study was to assess the suitability of chitosan nanoparticles (CNPs) for improving the antigen uptake by different antigen presenting cells to further enhance tumor-directed immune responses. CNPs can be used as carriers for noninvasive drug delivery, such as nasal or pulmonary administration. Hence, the uptake of FITC-conjugated CNPs of different sizes by human DCs or macrophages was analyzed by flow cytometry revealing a high uptake rate by DCs and a less efficient uptake by macrophages. Furthermore, to confirm intracellular CNP-localization in DCs, ImageStream® analysis was performed showing a high internalization rate of nanoparticles. In order to further investigate the suitability for inhalation, target specificity of CNPs was studied. Nanoparticle uptake analyzed in DCs, which were cocultured with human lung epithelial cells H441, showed CNP-uptake by both cell types. Current studies investigate the uptake of antigen loaded CNPs in cocultured DCs and whether this leads to potent T cell activation.

Overall, these data indicate that CNPs are efficiently internalized in DCs supporting their suitability as vehicles to improve antigen uptake by human DCs.

P.1.B1.08.01
Natural compounds as modulators of regulatory T cell function for the treatment of cancer
F. Al-Naimi, L. Theole, J. Bartel, M. Guderian, M. Swallow, L. Almedia, T. Sparrowasser; Institute of Infection Immunology, TWINCORE, Centre for Experimental and Clinical Infection Research, Hanover, Germany.

Regulatory T cells (Tregs) play a major role in maintaining the immune homeostasis of the host. However, their increased frequency observed in tumor patients and murine tumor models is associated with poor prognosis and inhibited therapy response rates. Therefore, their manipulation still is a major focus of current research. In recent projects we could show that natural compounds derived from micro-organismal secondary metabolites can influence the function of immune cells, especially T cells. In this project we identified a new additional compound that shows promising potential to inhibit regulatory T cells. First data indicated that this compound inhibits the differentation of Tregs in vitro by dampening the levels of FoxP3 protein, but not FoxP3 mRNA levels. Moreover, the compound did not impair the expression of IFNγ in Th1 polarized CD4 T cells in vitro. Furthermore, in vivo experiments showed that the therapy application of this compound in mice reduced the tumor growth rate, decreased Treg frequencies in the tumor-infiltrating lymphocyte (TILs) population and increased the CD8/Treg ratio in the TILs. Besides, RNA microarray analysis of iTregs treated with the compound further supports the idea that the compound regulates Fox3 on a posttranscriptional or even posttranslational level. Moreover, first human data in vitro induced Tregs from healthy donors cultured in the presence of the compound showed a decrease of Fox3 protein levels. Therefore, we propose that this new compound has promising immunomodulating capacity on Tregs useful for immunotherapy of melanoma.
POSTER PRESENTATIONS

P.B1.08.02
CD74, invariant chain, a modulator of endosomal maturation
G. Bakke1, S. Walchli1
1Department of Biosciences, Oslo, Norway; 2Sebastien Walchli, Oslo, Norway.

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CD74, with its two leucine-based endosomal sorting signals that binds AP1 and AP2 adaptors was first found to be an essential partner for the proper trafficking of MHC II and efficient antigen loading. The trimeric CD74 delays further endosomal maturation and is essential for forming the peptide loading compartment. CD74 has endosomal membrane fusion properties independent of Rab5, PI3 kinase and EAAT and I will discuss how this property can be exploited to study maturation, endosomal fusion, and endosomal membrane kinetics after tyrosine kinase receptor activation. Interestingly, CD74 has been found to interact with several molecules including MHCII and can be used as a vector for simultaneously increasing both MHCII and MHCII mediated immune responses towards specific antigens and is ready to be tested in clinical therapeutic DC based cancer immunotherapy.

P.B1.08.03
Targeting myeloid derived suppressor cells with all-trans retinoic acid is highly time-dependent in therapeutic tumor vaccination
A. Heine1, C. Flores1, H. Gevensleben1, L. Dieth1, M. Heikenwälder1, M. Ringelhart1, K. Janssen1, U. Nitsche1, N. Garbi1, P. Brossart1, T. Baumann1, P. A. Knolle1, S. Kurs1, B. Häcker1
1Medical Clinic III for Oncology, Hematology and Rheumatology, University Hospital, Bonn, Germany; 2Institute of Experimental Immunology, University Bonn, Bonn, Germany; 3Institute of Pathology, University Bonn, Bonn, Germany; 4Institute of Experimental Immunology and Hematology, University Medical Center, Hamburg-Eppendorf, Germany; 5Institute of Molecular Medicine, University Bonn, Bonn, Germany; 6Institute of Virology, Technische Universität München, Munich, Germany; 7Division of Chronic Inflammation and Cancer, German Cancer Research Center, DKFZ, Heidelberg, Germany; 8Department for Internal Medicine 2, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; 9Department of Surgery, Technische Universität München, Munich, Germany; 10Institute of Molecular Immunology, Klinikum rechts der Isar, Munich, Germany.

Therapeutic protocols aim to improve adaptive immune responses present a promising anti-tumor therapy. However, T cell mediated effector functions are often counteracted by regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs). Here, we studied therapeutic vaccination in two different mouse tumor models, B16-OVA (melanoma) and MC38-CEA (colorectal carcinoma).

Combined application of the TLR9-ligand CpG and the NK-IgG induced potent anti-tumor responses in only a subpopulation of mice. Non-responders had equal levels of Treg, CD4+ and CD8+ T cells, B cells and NK cells but elevated levels of CD11b+ MHCII- Ly-6G+ Ly-6C+ monocytes (MDMDCS). On a per cell basis, M-DMDCS from responders and non-responders were equal suppressive towards T cells. To overcome M-DMDCS mediated suppression, concurrent and time-delayed injection of all-trans-retinoic acid (atRA) was treated, a treatment previously reported to reduce M-DMDCS numbers. Strikingly, only 3d time-delayed but not concurrent administration of atRA reduced tumor growth and improved overall survival. M-DMDCS up-regulate MHCII, cross-present antigens and lost their suppressive effect towards T cells. In contrast, simultaneous treatment with atRA reduced the beneficial effects of therapeutic vaccination and did not lead to MHC class II expression. Similarly, MDSCs from human colorectal carcinoma patients failed to up-regulate HLA-DR after TLR stimulation in vitro when atRA was present. We hypothesize, treatment with atRA abrogates functional and phenotypic plasticity of myeloid cells. Therefore, timing of atRA administration should be carefully considered in therapeutic vaccination protocols to prolong a TLR-induced pro-inflammatory myeloid phenotype and to reduce immune suppression mediated by MDSCs.

P.B1.08.04
Sensitive identification and advanced profiling of neoantigen-specific T cells in cancer patients
S. Babice1, R. Genolet2, B. J. Stevenson3, V. Bianchi4, V. Zoeter4, D. Gijlers1, L. Kandalaft1, G. Couss1, A. Harari1
1Ludwig Institute for Cancer Research, Lausanne, Switzerland; 2Swiss Institute of Bioinformatics, Lausanne, Switzerland.

Neoantigens arise from tumor nonsomatic mutations and can generate immunogenic peptides (neo-epitopes) bound to HLA molecules. Mounting evidence suggests that neoantigens represent ideal tumor-specific targets to be exploited in T cell mediated immunotherapies or vaccines and several studies investigated demonstrated their clinical relevance. However, feasibility in low mutational load tumor types such as ovarian cancer remains unknown. Using highly sensitive assays to detect and profile neo-epitope specific circulating and tumor-infiltrating (TIL) CD8+ T cells allowed prompt identification of oligoclonal and polyfunctional such cells from most immunotherapy-naive patients with advanced epithelial ovarian cancer. Neo-epitope recognition was discordant between circulating T cells and TILs, and was more likely to be found among TILs, which displayed higher functional avidity and unique TCRs with higher predicted affinity than their blood counterparts. Of interest, the relative functional avidity of neoepitope-specific TILs correlated significantly with their intratumoral frequency. Our results imply that identification of neo-epitope specific circulating CD8+ T cells is achievable even in tumors with relatively low neo-epitope specific mutations, and neo-epitope identification in TILs extends opportunities for mutanome-based personalized immunotherapies to such tumors.

P.B1.08.05
HLA ligandomics feeds a pipeline of soluble T cell receptor-based immunotherapies
F. Capuano, G. Mommers, R. Carreira, D. Lowne, M. Cundell, A. Powlesland; Immunocore Ltd, Abingdon, United Kingdom.

Introduction: HLA complexes on cell surfaces can present cancer-associated peptides to the immune system, representing valuable targets for cancer immunotherapy. Immunocore identifies tumour-specific HLA-peptides, generates peptide-specific T cell clones and engineers T cell receptors (TCRs) into potent soluble immunotherapeutics.

Materials and Methods: Combinatorial protein microarrays of reconstituted HLA complexes were produced in lysis buffer and HLA complexes captured from the supernatant by affinity purification using HLA-restriction specific antibodies. HLA peptides eluted under acidic conditions are separated by reverse phase liquid chromatography and analysed by mass spectrometry. Fully validated target peptides were used to clonally expand HLA-peptide specific T cells. Isolated TCRs are engineered into soluble molecules (mTCRs) whose affinity towards target peptide:HLA is enhanced using phage display technology. Results: Our HLA peptidomic workflow combines novel biochemical techniques with high resolution mass spectrometry, data integration from multiple instruments and multiple search algorithms to maximise the depth of the HLA ligandome (up to 400,000 unique HLA-A*02 peptides identified). Our immune-activating therapeutics (ImmTAC") generated by coupling high-affinity mTCRs with an anti-CD3 scFv domain redirect polyclonal T cell responses toward cancer cells. Our current lead candidate, IMCgp100, has entered pivotal monotherapy trials for treatment of patients with metastatic uveal melanoma. Additionally, Immunocore in collaboration with MedImmune/AstraZeneca is conducting a trial in cutaneous melanoma patients exploring the combination of IMCgp100 with checkpoint inhibitors, including ImfinziTM (durvalumab) and tremelimumab.

Conclusions: Our peptidomics workflow enables the identification of HLA-presented peptides to support the successful design of TCR-based immunotherapies for cancer treatment.

P.B1.08.06
ERAP1 controls the engagement of human NK cell receptor KIR3DL1 by its specific ligand HLA-B51
M. Compagnone1, V. D’Alencar1, P. Guasp1, L. Cifaldi1, P. Romanò1, G. Ziccheddu1, V. Lucanini1, O. Melaiu2, D. Pende1, A. Harari1, A. López de Castr1, D. Fruci1
1Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy; 2Centro de Biología Molecular Severo Ochoa, CSIS, Madrid, Spain; 3IRCUS ADU San martino-Ist, Genoa, Italy.

The activity of natural killer (NK) cells is tightly regulated by inhibitory and activating receptors. Inhibitory killer immunoglobulin-like receptors (KIRs) survey the surface of target cells by measuring the expression of HLA class I (HLA-I) molecules. The interaction of KIRs with HLA-I molecules is sensitive to the sequence of peptides bound to HLA-I molecules, suggesting that NK-cell activation may be regulated by a change in the repertoire of peptide. Recently, we have demonstrated that genetic or pharmacological inhibition of ERAP1, a key component of the antigen processing, on tumor cells perturbs the engagement of NK cell inhibitory receptors by their specific ligands, enhancing the NK-mediated killing. These results indicate that modulation of ERAP1 can be exploited as a novel tool to improve the efficacy of NK-based approaches for cancer immunotherapy.

To determine the HLA-I/KIR combinations most affected of KIR ligands (HLA-A*01, -B51, -Cw3, -Cw4 and Cw7) were stably silenced for ERAP1 expression and tested for their ability to induce NK-cell degranulation. HLA-I surface expression did not substantially change following inhibition. Conversely, CD107a expression was significantly upregulated on NK cells following stimulation with ERAP1-deficient 721.221-B51 cells as compared with control cells. This result was particularly evident in the NK cell subset expressing the single KIR3DL1, and directly related to the functional level of this receptor. Overall, these results identify KIR3DL1/HLA-B51 as one of the most sensitive combinations to ERAP1 inhibition rendering tumor cells more susceptible to NK-mediated killing.
P.B1.08.07
Role of CD27/CD70 deficiency in the cell-mediated immune control of the Epstein Barr virus
Y. Deng, B. Chatterjee, C. Müns; Experimental Immunology, Zurich, Switzerland.

Introduction: Epstein Barr virus (EBV) is one of the most successful pathogens in the human population, persistently infecting more than 90% of adult individuals. It was discovered as the first human tumor virus contributes to 1-2% of all cancers in the world. Primary immunodeficiencies that predispose for EBV induced tumors and uncontrolled virus infection, have identified molecules in the differentiation, co-stimulation and effector function of cytotoxic lymphocytes. In this project, we are assessing CD27 immunodeficiency in EBV negative patient control and investigating which immune compartment(s) are compromised in the absence of CD27/CD70 co-stimulatory signal.

Material and Methods: To mimic the CD27 deficiency, anti-CD27 blocking and depletion antibodies were used to study the function of this pathway in EBV infection using T cell proliferation and killing assays in vitro. By reconstituting human immune system in mouse, the human-tropic EBV infection was able to be modeled in vivo. Conditions with/without CD27 injection were compared in terms of the EBV viral load and tumor incidence. In addition, the functionality of T and NK cells and cytokine production were monitored by flow cytometry.

Results: An impaired proliferation of T cells was observed due to the blocking effect of CD27 antibodies after 7 days of incubation. In vivo study showed depletion of CD27+ cells in mice led to significant higher EBV viral loads and a slightly increased incidence of tumorigenesis

Conclusions: Overall, CD27+ cells play a protective role in mediating EBV associated immune responses in humanized mice.

P.B1.08.08
Combination of Trastuzumab and Pertuzumab binding site mimotopes together with anti-PD1 Immune checkpoint as a novel anti-Her-2/neu multi-level vaccine
J. Tabios, M. Drinic, K. Baer, K. Ambroz, C. C. Zeliodor, U. Wiedermann; 1Institute of Hematology and Oncology, Medical University Vienna, Vienna, Austria, 2Division of Oncology, General Hospital Vienna., Vienna, Austria.

Extracellular subdomains of Her-2/neu is overexpressed in 20-25% of breast and gastric cancers. Combination of the mAbs Trastuzumab and Pertuzumab has synergistically resulted in a significant improvement in clinical outcomes of patients with Her-2/neu-positive metastatic breast cancer. However active immunotherapy, unlike application of mAbs, provides advantages like induction of humoral, cellular and memory responses and anti-tumor activity. The first generation of our B cell multi-antigen anti-Her-2/neu vaccine, containing threonyl peptides conjugated to virosomes, was recently improved to its second generation by fusing the peptides into a hybrid peptide (Pe6/1) which after conjugation to CRM197 and together with the adjuvant Montanide led to induction of strong humoral and Th1-biased cellular responses with antitumor activity. To broaden the number of biologically active epitopes in the vaccine, its third generation has recently been developed by including the binding site epitopes (mimotopes) of Trastuzumab and Pertuzumab, which in combination with Pe6/1 have shown to induce polyclonal humoral and cellular responses. Combinational therapy of cancer vaccines and immune checkpoint inhibitors has been suggested to synergistically enhance antitumor immune responses. When immunizing mice with Pe6/7-CRM-Montanide combined with anti-mouse PD-1 mAb vaccination, we have seen generally elevated cellular responses compared to unvaccinated mice. The third generation of our anti-Her-2/neu vaccine combined with an immunne checkpoint inhibitor is now being evaluated in vivo and may result in an effective novel multi-level vaccine against Her-2/neu overexpressing cancer entities. The study is granted by Imungene Limited, Australia, and Medical University of Vienna.

P.B1.08.09
LIGHT/LTBR signaling regulates self-renewal and differentiation of hematopoietic and leukemia stem cells
S. Hoepner1, R. Rodlour2, M. Amrein1, C. Riether1, G. Baertlocher3, A. Ochsenbein1; 1Inserm, Bern University Hospital/Dept. Medical Oncology/Tumor Immunology, DMMB, University of Bern, Bern, Switzerland, 2Institut, Bern University Hospital, Department of Hematology, Bern, Switzerland.

Introduction: Hematopoietic stem cells (HSC) are responsible to replenish all blood cell lineages. The balance between self-renewal, proliferation and quiescence is tightly regulated to ensure the maintenance of the stem cell pool but also to guarantee rapid adaptation in response to hematopoietic stress. Likewise, the balance of self-renewal and differentiation is critical in the pathology of hematopoietic malignancies. Here we show that lymphotoxin-beta receptor (LTBR), a member of the TNF superfamily, and its ligand TNFSF14 (LIGHT) play an important role in HSC and leukemic stem cell (LSC) regulation. LIGHT/LTBR signaling maintains stem cell quiescence, promotes symmetric division and thereby contributes to HSC and LSC self-renewal.

Material and Methods: To study LIGHT/LTBR signaling, we performed serial competitive repopulation assays by using hematopoietic stem and progenitor cells (HSPCs) from Ltbr KO, Ltbr KO of specific RT (WT) mice and analyzed cell cycle activity, cell viability and cell division. In a second approach we analyzed LTBR signaling in HSCs after induction of genotoxic stress. Therefore, S-Fluorocil (S-FU), a therapeutic agent which is known to activate HSCs, was administered into Ltbr KO and WT mice. Moreover, we investigated LIGHT/LTBR signaling in human HSPCs.

Results: Ltbr deficiency on murine HSCs and LSCs resulted in enhanced proliferation, asymmetric division and a reduced stem cell pool. LTBR signaling was induced by LIGHT in an autoocrine manner. Moreover, LTBR deficiency affected the sterness of human HSPCs.

Conclusion: LIGHT/LTBR signaling is a novel player in HSC and LSC self-renewal, which potentially provide a new strategy to eliminate LSCs.

P.B1.08.10
Pancreatic and colon cancer stem cells evade NK cell effector function through PCNA-NKp44 interaction
J. D. Malaer, P. A. Mathew; UNT Health Science Center, Fort Worth, United States.

Introduction: NK cells participate in the innate immune response against infection and cancer without prior sensitization. NK cell function depends on a balance of signals transmitted from activating and inhibitory receptors interacting with ligands on the surface of cells. Cancer cells may evade NK-mediated killing by expressing ligands for inhibitory receptors. Proliferating cell nuclear antigen (PCNA) associates with HLA I and forms the inhibitory ligand for NKp44, resulting in the inhibition of NK function. Cancer stem cells (CSC), a unique subset of tumor cells, possess a stem-cell like phenotype and are thought to facilitate metastasis by escaping NK cell effector function.

Materials and Methods: To study the mechanism of PCNA-NKp44 interaction, we performed blocking assays using antibodies against PCNA, NKp44 and CD133. To study the effect of PCNA-NKp44 interaction on NK cell function, we used NK cells isolated from human donor blood and NK cells isolated from 10 mice bearing Panc-1 xenografts.

Results: pcna−/− cells blocked the lytic activity of NK cells and CD8+ T cells but did not block the lytic activity of NK cells and CD8+ T cells in the presence of PCNA-NKp44 blocking antibodies.

Conclusion: Pancreatic and colon cancer stem cells evade NK cell effector function through PCNA-NKp44 interaction.

P.B1.08.11
Neoadjuvant therapy with attenuated Salmonella improves outcome of dacarbazine treated melanoma bearing mice
S. Chillbrose, A. E. Mônaco, M. Vola, C. I. Agoria, J. A. Chabalgoity, M. Moreno; Universidad de la República, Montevideo, Uruguay.

Melanoma is a severe form of skin cancer with high incidence rate. After 30 years of use, dacarbazine (DTIC)-based chemotherapy continues to be the standard of care for most patients with metastatic melanoma. In this work, we evaluate the potential of Salmonella enterica serovar Typhimurium, LVR01 (aroC), as neoadjuvant therapy in melanoma-bearing mice undergoing chemotherapy. C57BL/6 mice were subcutaneously inoculated with 1x10^6 melanoma cells. When tumors were palpable, S. Typhimurium LVR01 (aroC CFU) was intratumorally injected. At the following day, chemotherapy treatment consisting in daily intraperitoneally application of 150 mg/kg/doses DTIC was started and continued for 3 days. Neoadjuvant LVR01 administration in chemotherapy-treated mice retarded tumor growth and prolonged overall survival compared to control and monotherapy-treated animals. Importantly, Salmonella treatment was well tolerated by mice undergoing chemotherapy, with less than 10% of weight loss. This combined approach increased expressions ofctl2, ccs2, ccs1 and celi1 mRNA levels in the tumor microenvironment, accompanied by tumor infiltrating neutrophils and cytotoxic lymphocytes with augmented activated status. Chemotherapy therapy induced a drastic reduction of secondary lymphoid organ size. Salmonella neoadjuvant treatment partially rescued absolute cell numbers in these compartments, and activated effector NK and CD8 T cells. In conclusion, Salmonella immunotherapy could be safely used in individuals under chemotherapy treatment.

This therapeutic approach implies activation of cytotoxic lymphocytes, resulting in longer survival. The use of attenuated Salmonella as a non-specific active immunotherapy in combination with standard chemotherapy could be considered as an interesting therapeutic strategy with close clinical application for patients with melanoma.
POSTER PRESENTATIONS

P.B1.08.12
Anti-CD137 antibodies enhance Daratumumab efficacy against multiple myeloma increasing ADCC effect in an immunodeficient mouse model reconstituted with human NK cells


1Clinica Universidad de Navarra, Pamplona, Spain, 2Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Pamplona, Spain, 3Navarra Institute for Health Research (IDISNA), Pamplona, Spain, 4Division of Medical Oncology, Hospital Costa del Sol, Marbella, Spain, 5Centro de Investigación Médica Aplicada (CIMA), Pamplona, Spain, 6Centro de Investigación Médica Aplicada (CIMA), Pamplona, Spain, 7Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain, 8Universidad Pompeu Fabra (UPF), Barcelona, Spain.

Daratumumab is an anti-CD38 mAb approved for multiple myeloma treatment. Antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells and macrophages is one of the mechanisms of action proposed for this drug. Preclinical data have shown that the anti-CD137 agonist antibody Urelumab increases NK-mediated ADCC effect exerted by other mAbs used in oncology, such as Rituximab, Cetuximab and Trastuzumab, and clinical trials combining Urelumab and Rituximab or Cetuximab are ongoing. Freshly isolated human NK cells from healthy volunteers do not express CD137 on the membrane, but CD137 is induced when the NK cells are co-cultured with a Daratumumab-coated CD138+ multiple myeloma-derived cell lines (MM1S or KSMB28). Moreover, NK cells from multiple myeloma patient bone marrows treated ex vivo with Daratumumab also up-regulate CD137 expression. Daratumumab addition to co-cultures of NK cells and MM1S or KSMB28 increases tumor cell death. However, cytotoxicity is not increased by the addition of Urelumab either in short-term (4h) or medium-term (18h) experiments. To study if Urelumab increases Daratumumab-mediated ADCC activity in vivo, we set up a tumor model based on the i.v. administration of a luciferase-transfected human myeloma cell line, human NK cells and Daratumumab to immuno-deficient NSG mice. In this model, i.v. administration of Urelumab 24 h after tumor reestablished the tumor growth and prolonged mice survival. Financial sources: Worldwide Cancer Research Foundation (15-1146); Fundación Española contra el Cáncer (GCB15152947MELE). Becas de Formación e Innovación. Junta de Andalucía

P.B1.08.13
The Wisott-Aldrich syndrome protein regulates antigen processing and presentation by dendritic cells to activate cytotoxic T cells

M. Olivera, M. Baptista, L. Westerberg

1Karolinska Institutet, Solna, Sweden, 2Institute for Virology and Immunobiology, Würzburg, Germany.

One promising cancer therapeutic today is to teach the patient’s own immune system to kill tumor cells. Specialized dendritic cells (DCs) can take up exogenous antigen, such as tumor antigens, and present peptides on MHC class I molecules to activate CD8+ cytotoxic T cells in a process called cross-presentation. Here, we examined the role of the actin regulator Wisott-Aldrich syndrome protein (WASP) in antigen sorting in DCs. We found that specific deletion of WASP in DCs led to marked expansion of CD8+ T cells, suggesting that antigen sorting is regulated by WASP. To address if we could use our findings to target antigen sorting in wildtype DCs, we used small molecule inhibitors for actin regulators such as WASP. We have identified one inhibitor that induced increased cross-presentation to wildtype DCs. We treated DCs with this inhibitor during antigen sorting ex vivo and injected the DCs into mice. We found that inhibitor-treated DCs induced higher proliferation of antigen-specific CD8+ T cells in vivo. Our data suggests that direct target of actin regulators may enhance DC-mediated immunotherapy.

P.B1.08.14
Using tumour origins to identify peptide vaccine targets in two independent contagious cancers

R. Owen, A. Gastaldello, S. Ramarathnam, A. Bailey, P. Skipper, T. Elliott, A. W. Purcell, H. V. Siddele

1University of Southampton, Southampton, United Kingdom, 2Monash University, Melbourne, Australia.

The Tasmanian devil harbours two distinct transmissible cancers, Devil facial tumour (DFT) 1 and 2. Both are spread by transmission of cells between individuals, threatening a vulnerable species. As these cancers are allografts, Major Histocompatibility Complex Component (MHC) and bound peptides are key to developing vaccine strategies. DFT1 originated in a Tasmanian devil, DFT2 in a mainland a Schwann cell, but the cellular origin of DFT2 is unknown. Here we identify the cellular origin of DFT2 and characterise the immunopeptidomes of DFT1 and DFT2 to identify antigenic peptides. Peptides from MHC class I were isolated in triplicate for each cell line using a devil anti-B cell line. Mass spectrometry was used to sequence peptides and generate whole cell proteomes for DFT1-IFNy, DFT2 and devil fibroblast cell lines. Peptides were identified by searching spectra against custom Tasmanian devil databases using ExPeaks. Gene ontology analysis was performed. DFT2 and DFT1 are enriched for similar nervous system processes. DFT2 expresses proteins associated with glial development and myelin components. Between 2243 and 6737 potential MHC class I peptides were identified for each cell line with a length preference for 9mers. We identified 61 and 55 peptides unique to DFT1-IFNγ and DFT2. The immunopeptidomes of two contagious cancers will be used for vaccine design and to identify the binding motifs of MHC alleles expressed by DFT1 and DFT2. Our data suggests that DFT2 is of a myeloid competent glial cell lineage and bears remarkable resemblance to DFT1, a finding significant for both vaccine design and fundamental understanding of transmissible cancers. The Leverhulme Trust (RPG-2015-203)

P.B1.08.15
Development of a novel in vitro screening method for cancer immunotherapy using genetically modified NK-92 cells: Dissection of NK cell-tumor cell interactions

D. Oxelsson, Ussar, E. Celii, M. Chrabak, E. Romen, E. Alici, A. D. Durli, T. Sulz

1,2National Research and Application Center, Sabancı University, Istanbul, Turkey, 3Faculty of Engineering and Natural Sciences, Sabancı University, Istanbul, Turkey, 4NSU Cell Therapy Institute, Nova Southeastern University, Fort Lauderdale, United States, 5Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden.

NK cell-mediated lysis relies on a balance among several activating and inhibitory receptors that either promote or dampen the killing upon receptor-ligand interactions. Here, we propose to develop a screening method based on genetic modification of NK cells upregulating a constant target at a single cell. As both the character of the tumor cell population and the cytolytic status of NK cells differ among patients, we use a tool that can be instrumental in developing patient-tailored immunotherapeutic approaches. Genes coding for 20 different NK cell surface receptors were lentiviral vector backbones for genetic modification of NK-92 cells. New cell lines, each overexpressing a specific receptor, were subjected to phenotyping and assessment of effector functions. The use of a colorimetric substrate for Granzyme B activity allowed us to optimize an easy-to-use and phenotypic status of NK cells differ among patients, such a tool will be instrumental in developing patient-tailored cancer immunotherapy approaches.

P.B1.08.16
Evaluation of intravenous immunoglobulin use analyzed in relation to diagnostic evidence levels

D. Garcia-Cuesta, M. Vilches-Moreno, A. Salguero-Oldo, M. San Jose, A. Sampale

1UCLV Hematology, Immunology and Genetics. Hospital Puerta del Mar, Cádiz, Spain, 2UCLV Hospital Pharmacy. Hospital Puerta del Mar, Cádiz, Spain.

Aim: To evaluate clinical indication of intravenous immunoglobulin (IVIG) in the last year in our hospital in relation of based guideline of immunoglobulin Therapy. Evidence category and strength of recommendation was indicated.

Method: IVIG treatment was prescribed for 145 patients (76 male / 69 female; median age 43±5 months - 90 years). Clinical data, evidence category (EC) and strength of recommendation (SC) for indications. Data was evaluated in relation to 2016 update of the Consensus Document of the American Academy of Allergy, Asthma and Immunology. Results: IVIG were prescribed in 71 patients (49%) for indications definitively benefit 19, Primary immune deficiencies (Ib/B) 27 Immune thombocytopenic purpura (Ia/A), 12 Guillain Barré Syndrome (Ib/B), 8 Kawasaki disease (Ia/A), 6 Chronic desmyelinizing polyneuropathy (1a/A), 3 Multifocal motor neuropathy. IVIG were prescribed in 58 patients (40%) for indications probably benefit 14 Secondary immune deficiencies (Iv/Di) 21 Mestonia graves (Ib/B), 1 Eartmyositis (Ia/A) (1), 1 relapsing remitting multiple sclerosis (Ia/A), 8 Intractable childhood epilepsy (1a/B), 1 acute disseminated encephalitis transplants, 2 post DI, 3 highly sensitized patients for renal transplantation (IV,D). IVIG were prescribed for indications that may provide benefit in 8 cases (5.5%): 2 autoimmune blistering skin disease (1a/A), 3 severe resistant atopic dermatitis, 1 severe asthma. IVIG were prescribed for indications with no clinical evidence of benefit in 8 (5.5%) patients.

Conclusions: Data demonstrate an appropriate use of immunoglobulin. Use in which no long effective benefit was demonstrated might be avoided.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 241
Bifunctional degraders, also referred to as proteolysis-targeting chimeras (PROTACs), are a recently developed class of small molecules. They were designed to specifically target endogenous proteins for ubiquitin/proteasome-dependent degradation and to thereby interfere with pathological mechanisms of diseases, including cancer. In this study, we hypothesized that this process of acute pharmacologic degradation might increase the direct MHC class I presentation of degraded targets. By studying this question, we contribute to an ongoing discussion about the origin of peptides feeding the MHC class I presentation pathway. Two scenarios have been postulated: peptides can either be derived from homeostatic turnover of mature proteins and/or from short-lived defective ribosomal products (DRiPs), but currently, it is still unclear to what ratio and efficiency both pathways contribute to the overall MHC class I presentation. We therefore generated the intrinsically stable model antigen GFP-SBL-F12 that was susceptible to acute pharmacologic degradation via the previously described degradation tag (dTAG) system. Using different murine cell lines as a proof of principle, we show here that the bifunctional molecule dTAG-7 induced rapid proteasome-dependent degradation of GFP-SBL-F12 and simultaneously increased its direct presentation on MHC class I molecules. A dosycyclic-inducible setting, we could further show that the stable, mature antigen was the major source of peptides presented in our system. This study is, to our knowledge, the first to investigate targeted pharmacologic protein degradation in the context of antigen presentation and our data point toward future applications by strategically combining therapies using bifunctional degraders with their stimulating effect on direct MHC class I presentation.
**POSTER PRESENTATIONS**

**P.B1.09.03**

Functional study of tumor infiltrating lymphocytes in a breast cancer patient: an approach to personalized medicine


1. Universitat Autònoma de Barcelona, Bellaterra, Spain, 2. Vall d’Hebron Institute of Oncology, Barcelona, Spain, 3. Instituto Oncológico Baselga - Quirón Hospital, Barcelona, Spain, 4. Ramón y Cajal University Hospital, Madrid, Spain.

Breast cancer is the most common of women cancers. Triple-negative BC (TNBC), negative for estrogen and progesterone receptor and HER-2 genes, represent a clinical challenge because they do not respond to endocrine therapy or other targeted agents. Studying of tumor infiltrating lymphocytes (TILs) is promising field because of their good correlation with breast survival, particularly those with high CD8/Treg ratio.

We have studied TILs from a TNBC patient to characterize the immune response. TILs were obtained from a core biopsy that was cut in serial slices and cultured. Molecular and functional phenotype of TILs was studied by immunostaining of TILs, cytokine release and suppression assays, all analyzed by flow cytometry. TCRs from TILs, before and after expansions, have been sequenced in order to find if there are monoclonal expansions. TILs were a mixture of populations in all cultures, different CD4/CD8 ratios were observed related with their location on the biopsy. This different distribution of CTLs and CD4 TILs also affected the immune mediators detected in the supernatant, i.e. higher presence of cytokine-specific proteins Granyme B and IFN&gamma; in cultures with CTLs dominance. No cytokine profile could be defined on cultures with predominant CD4 T cells. TILs were expanded in vitro and tested in standard regulation assays. Cultures with predominant CTLs showed less capacity of inhibiting alloreactive proliferation compared with CD4 T cell cultures.

We have observed a heterogeneous distribution of TILs in the biopsy that may be useful to select the appropriate T cells to design tailored approaches to TNBC treatment.

**P.B1.09.04**

Heterologous prime-boost vaccination protects from Epstein-Barr virus antigen-expressing lymphomas

J. Rüff, C. Citterio, C. Leung, C. Münt;

1. Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland, 2. Nuffield Department of Medicine, Ludwig Institute for Cancer Research, University of Oxford, Oxford, United Kingdom.

Epstein-Barr virus (EBV) is a γ-herpesvirus that preferentially infects B cells and establishes lifelong chronic infection in more than 90% of the adult human population worldwide. The virus-host balance is mainly sustained by T-cell responses, which are able to control the infection asymptotically. However, when T-cell immune control fails, EBV is able to establish a latent infection or of human malignant transformation of human nasopharyngeal carcinoma. EBV is one of the predominant tumor viruses in humans, but so far no therapeutic or prophylactic vaccine against this transforming pathogen is available. We demonstrate that heterologous prime-boost vaccination with the viral antigen 1 of EBV (EBNA1) either targeted to the DEC205 receptor on dendritic cells or expressed from a recombinant modified vaccinia virus Ankarla (MVA) vector induced a strong priming of antigen-specific CD8+ T cell help. CD4+ T cell help supports the expansion and maintenance of EBNA1-specific CD8+ T cells that are most efficiently primed by recombinant adeno-viruses that encode EBNA1. These combined CD4+ and CD8+ T-cell responses protect from EBNA1-expressing T-cell lymphomas and B-cell lymphoproliferations that emerge spontaneously after EBNA1 expression. Especially the heterologous EBNA1-expressing adeno-virus, boosted by EBNA1-encoding MVA vaccination demonstrated protective effect as prophylactic and therapeutic treatment of the respective lymphoma challenges. Therefore, we propose that such heterologous prime-boost vaccinations should be further explored for clinical development against EBV-associated malignancies as well as symptomatic primary EBV infection.

**P.B1.09.05**

Live cell imaging of lytic granule motility in anti-ErbB2 NRK cells and FRK NRK cells plus Herceptin towards ErbB2+ breast cancer cells

N. Worthco1, 2, J. Eitter1, 2, T. Müller-Reichert1, M. Gerlach1, W. Wells1, T. Tone1, 3, H. G. Klingemann;

1. Experimental Transfusion Medicine, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, 2. Institute for Transfusion Medicine Dresden, German Red Cross Blood Donation Service North-East, Dresden, Germany, 3. Partner Site Dresden, Dresden, Germany, 4. Structural Cell Biology Group, Experimental Center, Medizinische Fakultät Carl Gustav Carus, University of Technology, Dresden, Germany, 5. Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt am Main, Germany, 6. German Cancer Consortium (DKTK), Partner Site Frankfurt/Mainz, Frankfurt am Main, Germany, 7. Nordwest inc., Culver City, United States.

Upon encountering a susceptible target, NK cells mediate directed cytotoxicity by exocytosis of lytic effector molecules such as perforin and granzymes. The steps leading to NK granule exocytosis are highly regulated. Granule exocytosis is preceded by convergence of granules to the microtubule organizing center (MTOC) and subsequent polarization of the MTOC and granules to the immunological synapse (IS). In case of antibody-dependent cell-mediated cytotoxicity (ADCC), it has been shown that signaling through the Fc receptor is critical to polarize MTOC and granules to the IS with otherwise resistant targets. Here we used spinning disk confocal microscopy for live cell imaging to analyze granule-mediated NKG2+ NK cell cytotoxicity in ErbB2-targeted CAR expressing NK-92 cells (NK-92/5.28.2) and research-grade high affinity FcR-expressing NK-92 cells plus Herceptin™ towards ErbB2-positive breast cancer cells (MDA-MB-453), which are resistant to parental NK-92. Interestingly, unmodified NK-92 cells in combination with MDA-MB-453 cells showed granule convergence to the MTOC, but failed to polarize MTOC and granules to the IS. In contrast, retargeting by either CAR or mAb/FcR towards the ErbB2 antigen on MDA-MB-453 enabled granule polarization to the IS resulting in highly effective cytotoxicity. Granule polarization was rapid in both the ErbB2 and high affinity FcR-expressing NK-92 cells after cell-cell contact was initiated (3 and 9 minutes respectively). These observations suggest that retargeting of NK-92 cells by either transgenic CAR or high affinity FcR expression in combination with tumor-specific antibodies confers tumor cell lysis by enabling the otherwise impaired FcR-mediated and granule polarization to the IS which resembles the physiological exocytosis cascade observed in naturally occurring ADCC.

**P.B1.09.06**

Activation of phosphorylation of STAT3 by farnesoid X receptor accelerates the migration of NSCLC cell induced by lung fibroblast

H. Jiang, X. Liu;

1. RENH HOSPITAL, SCHOOL OF MEDICINE, SHANGHAI JIAO TONG UNIVERSITY, SHANGHAI, China.

Objective: We aimed to study that whether the bile acid nuclear receptor (FXR) played a role in the complex dynamic interaction between cancer associated fibroblasts (CAFs) and lung cancer cells. Materials and methods: Human lung cancer cells (AS49 cells) were co-cultured with lung fibroblasts (HFP cells) for 48 hours in vitro. The expression of proteins, the migration abilities and the levels of cytokines were detected by western blot assay, the transwell migration assay and cytokines antibody arrays respectively. Results: Western blot results showed that the expression of FXR and STAT3 phosphorylation (at Tyr705) in the AS49 cells increased in the co-culture system. The transwell migration assay showed that the cell migration ability in the AS49 cells was also improved compared with the control group (P<0.001). Meanwhile, the levels of some cytokines such as TNF RII, IL-17B and RGM-B were significantly up-regulated in the co-culture supernatant (P<0.05). The levels of TNF RII, IL-17B and RGM-B increased 18.2, 11.3 and 8.7 times as high as the controls respectively. However, these effects could be reversed by silencing FXR with si-RNA in AS49 cells (P<0.05). Conclusion: FXR accelerates to the migration of NSCLC induced by lung fibroblast-tumor cells interaction though STAT3 signaling pathway.

**P.B1.09.07**

Neutral sphingomyelinase 2 (nSMase2) promotes T cell infiltration in colorectal tumors and promotes immune checkpoint inhibitors activity


1. INSERM U1037, CRCT, Université de Caen, Caen, France, 2. INSERM U1037, CRCT, Université de Caen, Caen, France, 3. Stony Brook University, Stony Brook, NY 11794, United States, 4. INSERM U1036, Dijon, France, 5. INSERM U1037, CRCT, Université Toulouse III, Toulouse, France, 6. INSERM U1038, Toulouse, France, 7. Experimental Transfusion Medicine, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, 8. Experimental Center, Medizinische Fakultät Carl Gustav Carus, University of Technology, Dresden, Germany, 9. Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt am Main, Germany, 10. German Cancer Consortium (DKTK), Partner Site Dresden, Dresden, Germany, 11. German Cancer Consortium (DKTK), Partner Site Frankfurt/Mainz, Frankfurt am Main, Germany, 12. Northwest Inc., Culver City, United States.

Background: neutral sphingomyelinase 2 (nSMase2) belongs to a network of sphingolipid-metabolizing enzymes. More specifically, it catalyses the hydrolysis of sphingomyelin into ceramide, a bioactive lipid considered as an anti-oncometabolite. Results: Gene expression analyses of melanoma tumours of the TCGA dataset revealed the gene coding for nSMase2, SMPD3, was expressed at lower levels in metastases as compared to primary masses. Moreover, high levels of the transcript coding for nSMase2 in tumour biopsies were associated with better overall survival for advanced melanoma patients. In the mouse B16 melanoma model, which displays low levels of nSMase2, overexpression of this enzyme did not affect cell growth under 2D condition, however, it decreased tumour growth in vivo. Surprisingly, nSMase2 overexpression increased the infiltration of tumours by CD8+ T cells and the nSMase2-dependent delayed tumour growth was abolished in CD8 KO mice. Mechanistically, increased nSMase2 activity led melanoma cells to secrete exosomes enriched for the immunogenic mir155, thus favouring dendritic cell activation and anti-melanoma CD8+ T cell responses in vivo. Finally, increased nSMase2 activity in tumours synergized with anti-PD-1 therapy to abolish melanoma growth in vivo. Conclusion: Our work highlights a new role for the sphingolipid metabolism in the modulation of the immune microenvironment of melanoma by malignant cells.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 243
In cancer therapy, immune cells can be used as delivery platform for highly toxic compounds like immunotoxins in order to reduce their side effects. Here we investigated the potential of human primary T cells to deliver immunotoxins. T cells were engineered by transient transfection with immunotoxin encoding mRNA. The recombinant toxin, secreted by T cells, was expressed in sufficient efficiency in an adoptive T cell therapy. Therefore two Pseudomonas exotoxin A-based immunotoxin constructs (e23-PE38 and VEGF-PE38) were designed. Ex vivo activated, primary T cells were transfected with in vitro synthesized mRNA, coding for e23-PE38 and VEGF-PE38. Successful expression and secretion of the immunotoxin were shown by Westernblot and sandwich-ELISA analyses. WST assay revealed that immunotoxin expression impaired the viability of transfected T cells. Nevertheless, in vitro toxicity tests showed that immunotoxin expressing T cells were able to perform residual effector functions, mediated by HEA125xOKT3 bispecific antibody. An additional effect of the immunotoxin was not observed. It was possible to restore impaired viability and reduced proliferation of transfected T cells by expressing an attenuated version of VEGF-PE38. MALDI-TOF analysis revealed that the immunotoxin was partially translocated into the endoplasmic reticulum. By expressing mRNA immunotoxins in T cells, we were able to modulate their functions through decreasing MSDC expression of immunosuppression-related genes in vitro.

The effect of vitamin E on the function and frequency of myeloid-derived suppressor cells in an experimental breast cancer model

P. B1.09.10

Y. Vogtig, S. Habibi, J. Hadjii, M. Vogtig;
Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Background: Vitamin E has been shown to have strong anti-carcinogenic properties, including antioxidant and apoptotic characteristics, making it appealing candidate for cancer therapy. On the other hand, among the tumor immuno suppressive components, it has been shown myeloid derived suppressor cells (MDSCs) have remarkable ability to suppress anti-tumor immunity through multiple mechanisms. The aim of current study is to assess whether the alpha-tocopherol succinate can alleviate MDSCs-mediated immunosuppression in vitro and in an experimental breast cancer model.

Material and Methods: After assessing the effect of alpha-tocopherol succinate on MDSC viability and gene expression in vitro, mice were challenged with 7x10^5 4T1 murine breast adenocarcinoma cells. After 5 days, tumor-bearing mice were intraperitoneally injected with vitamin E (5mg/kg) or DMSO/Tween (vitamin E solvent) at one day interval for a total of five times. After isolation of MDSCs from the spleen and tumor tissue, MDSCs frequency, nitric oxide (NO) production and gene expression analysis were performed by flow cytometry and quantitative RT PCR respectively.

Results: Based on our experiments, vitamin E diminished tumor growth and extended survival in tumor bearing mice but it had no effect in tumor bearing mice but it had no effect on MDSCs frequency in tumor bearing mice however it may modulate their functions through decreasing MDSC expression of immunosuppression-related genes in vitro.

Unbiased identification of CD4 T-cell epitopes using novel MHC-based chimeric receptors

P. B1.09.11

J. Kisielow, F. Obermaier, M. Kaup;
Institute of Molecular Health Sciences, ETH, Zurich, Switzerland.

αβ T-cell receptors (TCRs) bind peptide-major histocompatibility complexes (pMHC) with low affinity, posing a considerable challenge for direct identification of αβ T-cell cognate peptides (epitopes). Here, we describe a platform for the discovery of MHC class ii presented epitopes, based on screening of engineered reporter cells expressing novel pMHC-TCR (MHC) hybrid molecules carrying αβ DNA-derived peptides. This technology identifies natural epitopes of CD4 T-cells in an unbiased and efficient manner and allows detailed analysis of TCR cross-reactivity providing recognition patterns on top of discrete epitopes. We identify cognate peptides of virus- and tumor-specific T-cells in mouse disease models and present a proof-of-concept for human T-cells. Furthermore, we show that vaccination with a peptide naturally recognized by TILs can efficiently protect from tumor challenge. Thus, the MCR technology holds promise for basic research and clinical applications allowing personalized identification of T cell antigens in patients.

Memory CD8 T cell infiltration promotes tissue-residency

P. B1.09.12

1LUMC, Leiden, Netherlands, 2University of Oxford, United Kingdom.

Memory T cell infiltration is a phenomenon occurring upon infection with certain chronic viruses that is characterized by the maintenance of large populations of circulating antigen-specific memory CD8 T cells with an effector-memory-like phenotype. Whether memory CD8 T cell infiltration is related to the formation and maintenance of tissue-resident memory (TRM) T cells is not known. Here we studied the induction and maintenance of CD8+ TRM T cells upon immunization with adenoviral vectors modified to elicit memory T cell responses in vivo, mice were challenged with 7x10^5 4T1 murine breast adenocarcinoma cells. After 5 days, tumor-bearing mice were intraperitoneally injected with e23-PE38 protein or the immunodominant epitope from E7 protein elicted E7-specific memory infiltration in a dose-dependent manner. Interestingly, E7-specific CD8+ TRM T cells were generated and maintained for months in multiple organs after vaccination, and the numbers of these E7+ TRM T cells associated with memory infiltration. The vaccine-induced CD8+ T cell responses conferred long-term protection in a mouse model of HPV-induced carcinoma, and this protection depended on the development of CD8+ TRM T cells. Moreover, this formation of CD8+ TRM T cells could be enhanced by temporal targeting costimulatory interactions early after immunization. Together, these data suggest that the induction of tissue-residency is linked to the memory infiltration, and can be enhanced by targeting costimulation.
Objective clinical responses were documented in 3 patients and among them 2 occurred in patients injected with cell products harboring two KIR ligand mismatches and one in a patient with one KIR ligand mismatch. Immune monitoring revealed that most patients presented an increased but transient of IL-15 and IL-7 cytokines levels one week after chemotherapy. Furthermore, a high expansion of FoxP3 regulatory T cells and PD-1+ T cells was observed in all patients, related to IL-2 administration. Our results demonstrated that combining allogeneic NK cell transfer via intra-hepatic artery, cetuximab and a high-dose IL-2 is feasible, well tolerated and may result in clinical responses.

P.B1.09.15
Antigen presentation to CD169+ macrophages: translation to the human situation and coporation to DC-SIGN targeting
M. López-Venegas1, A. Barbacid1, K. Oleske1, L. Hoogen1, M. Ambrosini1, H. Kolay1, G. Sturm2, E. Puchhammer-Stöckl2, Y. van Knoon1, J. den Haan1;
1Amsterdam UMC, Amsterdam, Netherlands, 2Utrecht University, Utrecht, Nethrlands, 3Medical University of Vienna, Vienna, Austria.

Dendritic Cell Specific Intercellular adhesion molecule 3-Grabbing Non-integrin (DC-SIGN) and CD169/Sialic acid binding immunoglobulin type lectin 1 (siglec-1), are lectin receptors expressed by macrophages in secondary lymphoid organs and are implicated in antigen uptake. Our aim is to compare the efficacy of these lectin receptors with regard to antigen uptake and cross-presentation by monocyte-derived DCs (moDCs).

Fluorescent DC-SIGN and CD169-specific antibodies or liposomes containing the DC-SIGN and CD169 specific ligands Lewis Y and monosialodihexosylganglioside (GM3) were used to target gp100 melanoma antigen to moDCs. Binding and uptake of our targeting strategies was investigated by flow cytometry, while imaging flow cytometry was employed to study antigen routing. The determination of gp100 cross-presentation was assessed by coculturing targeted moDCs with gp100 specific HLA-A201 restricted T cells and analyzing IFNγ production. Our preliminary data show that liposome and antibody targeting of gp100 to DC-SIGN and CD169 lead to effective binding, but also suggests that DC-SIGN and CD169 ligand receptors may have a differential capacity to endocytose and to stimulate cross-presentation of antigens. Our studies will help to determine which lectin receptor is the most efficient to target antigens to for the activation of anti-melanoma T cell responses.

P.B1.09.16
Robust GMP manufacturing process with IL-2, fibronectin and anti-CD16 antibodies generates highly active human NK cell batches for cancer immunotherapy
K. Bröker1, U. Schumacher1, R. Pirten2, H. Hoffmeister3, E. Sinekina3, S. Lüth4, W. Dammermann3;
1Brandenburg Medical School, Brandenburg a. d. Havel, Germany, 2University Medical Center Hamburg-Eppendorf, Hamburg, Germany, 3Hamburg University of Technology, Hamburg, Germany, 4Zellwerk GmbH – HiPer-Group, Oberkrämern, Germany.

Introduction: NK cells are innate immune cells crucial for killing of infected and malignant cells. They are able to fight circulating tumor cells thereby preventing metastases formation which account approximately for 90% of all cancer deaths. Thus, NK cells became interesting candidates for cancer immunotherapy and ex vivo manipulation and expansion of highly potent NK cells as a promising tool for adoptive transfer in patients an urgent need.

Methods: Human, CD3+ T cell-depleted PBMCs were expanded in a fibronectin and anti-CD16 antibody-coated bioreactor in presence of IL-2 following GMP guidelines. Cells were analyzed for NK cell purity, expression of different chemokine receptors, cell adhesion molecules, activating receptors and death ligands as well as IFNγ production using flow cytometry.

Further cytotoxicity towards different tumor cell lines was assessed via LDH assays and flow cytometry-based degranulation assays.

Results: Upon expansion NK cell purity reached 95% to 96%. The cells showed expression of the chemokine receptors CXCR3, CXCR4 and CCR5 and the cell adhesion molecules L-selectin, LFA-1 and VLA-4. Further, they expressed the activating receptors NKP30, NKP46, NKP44, NKG2D, DNAM-1 and CD16, the death ligands Fas and TRAIL and produced IFNγ.

Conclusion: We describe a novel approach for ex vivo NK cell expansion generating a set of highly potent NK cells which represent promising candidates for cancer immunotherapy. The GMP manufacturing process allows the use of these cells in clinical trials, i.e. adoptive NK cell transfer in patients.

P.B1.09.17
TNFα blockade overcomes resistance to anti-PD1 in experimental melanoma
A. Montfort1, F. Bertrand1, E. Marcheteau1, C. Imbert1, J. Gilhet2, T. Fillon1, P. Rochat1, N. Andrieu-Abadie1, T. Levalle1, N. Meyer1, M. Ambrosini1, C. Colacios2, B. Ségal1,2;
1INSERM U1037, CRCT, Toulouse, France, 2Institut Universitaire du Cancer (IUCT), Toulouse, France, 3Université Toulouse III, Toulouse, France, 4CHU Purpan, Toulouse, France.

Anti-PD1 therapy has significantly improved the care of melanoma patients. However, about 40 to 70% of them do not display optimal response to treatment and responders often relapse or experience mild to severe immune related adverse events (irAEs). While anti-Tumor Necrosis Factor α (TNF) antibodies were successfully used in the clinic to help control irAEs, their impact on the anti-tumor activity of immune-based IL-2 therapies remained unknown. Our pre-clinical studies demonstrated that blocking the TNF/TNFRI pathway potentiated the CD8+ dependent anti-melanoma immune response in mice. Moreover, blocking the TNF/TNFRI pathway synergized with anti-PD1 treatment to impair tumor growth in mice. In this context, we found anti-TNF prevented the anti-PD1 dependent upregulation of TIM-3 on T cells as well as activation-induced cell death thus favoring CD8+ T cell accumulation in tumors. These results lead our team to take part to a phase 1b clinical trial aiming at evaluating the safety and tolerance of combining immune checkpoint inhibitors (ICI) to anti-TNF by metastatic melanoma patients (TICIMEL: NCT03293784).

P.B1.09.18
A non-small cell lung cancer (NSCLC) mouse model for improved preclinical validation of immune therapies
M. Schuem1; Institut für Translationale Immunologie, Mainz, Germany.

Introduction: Lung cancer has an overall dismal prognosis and only when diagnosed early, surgery and ablative therapies may offer a cure.Checkpoint inhibitors are a first generation alternative treatment option promising objective response rates and in some cases complete tumour remission. The failure of established treatment strategies and the therapeutic benefit of immune therapies highlight the need for additional research with tractable models.

Methods: For preclinical testing of immune therapies, we have generated a mouse model containing a conditional gene switch that sets off oncoenogene K-Ras12v and inactivates p53 is the Kras12v system. We designed the model to allow for the conditional induction of SKP2 in the mouse lung. The model also contains two conditional reporter genes (EYFP and lacZ) to trace transformed lung cancer cells. Finally, we established several lung cancer cell lines from primary SKP tumours.

Results: Immune response (TIM) injection in SKP mice caused durable regression of lung tumor progression and the co-expression of EYFP and lacZ reporter genes. The specific expression of reporter genes in malignant cells allowed to exactly quantify the therapeutic benefit of different therapies/immune therapies. Injection of tumor-derived cell lines demonstrated that these cells metastasize to the brain, the peritoneum and the liver.

Conclusion: With the autochthonous SKP model and its metastasized SKP cell line derivatives any experimental immune therapy can be tested in an adequate and reliable preclinical setting. Co-expression of the two reporter genes differentiates between transformed tumor cells and non-malignant tumor-constituting cells thus allows to study the effect of different therapies for malignant tumor cells and non-transformed bystander cells.

P.B2.01 Environmental regulation anti-tumor responses - Part 1
P.B2.01.01
Assessment of promoter hypermethylation in tissue and blood of non-small cell lung cancer patients and association with survival
A. Ali 1, S. Sohal, U. Udhayathay, A. Mohan, K. Madan, K. Luthra, S. Kumar, W. Rafi, R. Guleria; All India Institute of Medical Sciences, New Delhi, India.

Background: Gene silencing by aberrant promoter hypermethylation is common in lung cancer and is an initiating event in its development. Aim To compare promoter hypermethylation frequency in serum and tissue of lung cancer patients with disease controls. Cyclin-dependent kinase inhibitor 2A (p16), a tumor suppressor gene, plays an important role in cell cycle regulation. O6-methylguanine DNA methyltransferase (MGMT) gene encoded protein is essential for genome stability.Method 95 newly diagnosed untreated advanced stage lung cancer patients and 50 cancer free matched controls were studied. Bisulfit modification of tissue and serum DNA was done; modified DNA was used as a template for methylation specific PCR analysis.

Survival was assessed for one year. Results Of 95 patients, 82% were non-small cell lung cancer (34% squamous cell carcinoma, 34% non-small cell lung cancer and 14% adenocarcinoma) and 18% were small cell lung cancer. Biopsy revealed that tissue of 89% and 75% of lung cancer patients and 85% and 52% of controls had promoter hypermethylated for MGMT (p<0.35) and p16 (p<0.001) gene, respectively. In serum, 33% and 49% of lung cancer patients and 28% and 43% controls were positive for MGMT and p16 gene. No significant correlation was found between survival and clinicopathological parameters. Conclusion High methylation frequency of p16 gene in tissue biopsy may be Initial with early stages of carcinogenesis. Appropriate follow-up is required for confirmation of this finding.

P.B2.01.02
Gene silencing by aberrant promoter hypermethylation is common in lung cancer and is an initiating event in its development.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.B2.01.02 Implication of matrix metalloproteinases 9/2 and nitric oxide in the development of breast cancer: correlation with clinicopathological parameters

M. Amiri1, A. Koukhar2, N. Benjedane3, C. Toulou-Biaskopf4
1University USTHB, Faculty of Biological Sciences, Laboratory of Cellular and Molecular Biology, Algiers, Algeria, 2Department of anatomic-pathology, Centre Pierre and Marie Curie, Algiers, Algeria.

Introduction: Many studies have demonstrated that nitric oxide (NO) plays a significant role in the multisite processing of carcinogenesis in breast cancer patients. These steps involve many inflammatory mediators like matrix metalloproteinases, in particular MMP-9 and 2. Thus, the aim of the present study was to investigate the activity levels of MMP-9, MMP-2 and NO in breast cancer patients.

Methods: MMP activities were assessed by a zymographic analysis in the sera of 125 patients carrying breast tumors and 20 healthy subjects as well as 6.3 breast tumors. Moreover, NO activity was investigated in the same samples by an enzymatic method. The results obtained were then correlated with the clinicopathological parameters. Moreover, immune-histochimical staining was performed to analyze the tissue expression of CCL6 (marker of infiltrating macrophages), uNOS (universal NO syntheses), and NFkB. Results: The activities of MMPs and NO increased significantly in breast cancer patients compared with control subjects. Moreover, these activities were higher in patients with malignant tumors than in those with benign tumors both in sera and biopsy. They may also correlate with tumors’ size, type, stage, metastasis, and tissue expression of uNOS, CCL6 and NFkB. Conclusion: Our results showed an association between high activities of MMPs (particularly MMP9) and NO and the development of breast malignant tumors. Interestingly, the serum MMP and NO level reflect the tissue levels. These findings suggest that serum levels of these molecules may be useful marker in monitoring breast carcinoma patients.

P.B2.01.03 Notch and Aiolos transcription factors in B CLL

J. Skelin1, L. Milivojevic1, I. Felicelovac1, B. Jelic Puskaric1, M. Maticul1, I. Kardum-Skelin2, D. Radio-Krstev3, M. Antic4
1Ruđer Bošković Institute, Zagreb, Croatia, 2Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi di Napoli Federico II, Naples, Italy, 3Department of Clinical Cytology and Cyto genetics, Merkur University Hospital, Zagreb, Croatia, 4University of Zagreb, Faculty of Science, Zagreb, Croatia.

Background: The notch signalling pathway is a complex biological network which promotes cell proliferation and determines cell fate. The notch ligands Delta-like (DLL) and Delta-like (DLD) ligands induce the activation of the Notch receptor and lead to the activation of the transcription factor Hes. Therefore, we established a coculture system with the Delta-like ligand transfected OP9 cells which is mandatory for T-cell development in order to explore the activation potential of the Notch receptors we found expressed also by B-CLL cells.

Conclusion: Although well explored in T-cell acute lymphoblastic leukemia, the Notch specific pathway involvement in B-CLL has been contradictory so far. In addition to Notch, and others have previously shown that a member of the Ikaros family of zinc-finger proteins, Aiolos has a very high expression in B-CLL lymphocytes and there is evidence suggesting its role in the survival of other leukemic B cells. We analysed these genes simultaneously and tested apoptosis regulation by Notch activation as co-cultures of the malignant cells on the OP9-DL1 cell line.

P.B2.01.04 Relation between dendritic cells and regulatory T cells in mammary neoplasm in dogs

P. H. L. Bertola1, M. C. Rosolem2, M. B. Conceição2, P. R. Moreira3, R. O. Vasconcelos2
1Sao Paulo State University (UNESP), Jaboticabal, Sao Paulo, Brazil.

Introduction: The malignant mammary tumors have several ways of evading the immune system, including the modulation of dendritic cells (DCs), by interfering with their maturation, resulting in inefficient presentation of antigens to T cells and consequent induction of immunological tolerance. Therefore, this study aimed to evaluate the relationship between DCs and regulatory T cells (Treg) in the simple type canine mammary tumors.

Results: A reduction in the percentage of circulating ILC subsets was observed in patients, as compared to healthy controls. The NK-cell phenotype showed alterations in the expression of the CD5 antigen together with specific B-cell antigens CD19, CD20 and CD23. We used this feature of the abnormal B-cell clone expressing CD5 and characterised the individual cell groups within the sample in the transcriptional and protein level in order to study the aberrant developmental stages of the B cells in this disease regarding the roles of Ikaros transcription factor Aiolos, the Notch signalling pathway and its target genes Hes and Delta. We also established a coculture system with the Delta-like ligand and transfected OP9 cells which is mandatory for T-cell development in order to explore the activation potential of the Notch receptors we found expressed also by B-CLL cells.

Conclusion: The accumulation of tumor produced CCL19 in the blood may promote the selective blood metastatic spread of the CCR7+ melanoma CSCs, which were highly susceptible to NK cells-mediated killing. Moreover, NO activity was investigated in the same samples by an enzymatic method. The results obtained were then correlated with the clinicopathological parameters. Moreover, immune-histochimical staining was performed to analyze the tissue expression of CCL6 (marker of infiltrating macrophages), uNOS (universal NO syntheses), and NFkB. Results: The activities of MMPs and NO increased significantly in breast cancer patients compared with control subjects. Moreover, these activities were higher in patients with malignant tumors than in those with benign tumors both in sera and biopsy. They may also correlate with tumors’ size, type, stage, metastasis, and tissue expression of uNOS, CCL6 and NFkB. Conclusion: Our results showed an association between high activities of MMPs (particularly MMP9) and NO and the development of breast malignant tumors. Interestingly, the serum MMP and NO level reflect the tissue levels. These findings suggest that serum levels of these molecules may be useful marker in monitoring breast carcinoma patients.
The influence of short-term and long-term ibritinib treatment on the HLA-DR expression on leukemic and T cells in chronic lymphocytic leukemia

G. Gabova1, Z. Mikuškova2, G. Manukyan1, T. Papajik1, P. Turcanyi1, R. Fillera1, T. Dyskova1, V. Smotkova Kraiczova1, S. Zehnalova1, P. Gajdor1, R. Urbanova1, M. Kudelka1, E. Kriegova2;
1Department of Immunology Faculty of Medicine and Dentistry Palacky University, Olomouc, Czech Republic, 2Laboratory of Molecular and Cellular Immunology, Institute of Molecular Biology NAS RA, Yerevan, Armenia

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.B2.01.12

Modulation of pulmonary microbiota by antibiotic or probiotic aerosol therapy: a new strategy to promote immunosurveillance against lung metastases


1Molecular Targeting Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, 2Dipartimento di Scienze degli Alimenti, Nutrizione e Ambiente (DeFENS), Università degli Studi di Milano, Milan, Italy, 3Immunotherapy Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, 4Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milan, Italy.

Immuno logical tolerance in the lung microenvironment is essential to control inflammation in response to inhaled particulates, but it also creates a permissive milieu for the setting of lung metastasis. Since the lung microbiota is implicated in this tolerance, we explored whether its manipulation via antibiotics or probiotics aerosolization in C57Bl/6 mice limits melanoma metastasis by subverting local immune suppression and boosting immune responses. Here, we show that in lungs of vancomycin/neomycin aerosolized mice a decrease in bacterial load was associated to a reduction in regulatory T cells (Tregs). Moreover, the enhanced activation of lung T and NK cells paralleled the significant reduction of lung metastases in mice aerosolized with antibiotics and intravenously injected with melanoma B16 cells. Reduction of lung metastases also occurred in lung transplanted with bacteria isolated from the bronchoalveolar lavage of antibiotic-treated mice (Morganella morganii, Campylobacter sp); likewise, transplantation of bacteria isolated from untreated mice (Porinbacillus glucanolyticus, Bacillus clausii) attenuated the anti-metastatic effects of antibiotics. Aerosolized probiotic Lactobacillus rhamnosus, a human commensal bacterium, strongly limited B16 metastases implantation and promoted immune activation as well. Furthermore, probiotics or antibiotics improved the therapeutic effects of dacarbazine, a chemotherapeutic agent used in metastatic melanoma patients, in advanced B16 metastases-bearing mice. Our results reveal for the first time that the balance of immune regulation of microbial bacteria in lungs is relevant in creating an immunological permissivity milieu for metastatization. Thus, targeting lung microbiota via probiotic or antibiotic aerosolization represent a new therapy to prevent metastases and enhance the response to chemotherapy.

P.B2.01.13

IgG subclass switching and clonal expansion in human colorectal cancer

R. Liu, Q. Zhang, H. Liu

Institute of Gastroenterology and the Sixth Affiliated Hospital, Guangzhou, China.

B lymphocytes play an important role in the maintenance of intestinal homeostasis. However, the subsets and roles of B lymphocytes in human colorectal cancer are not clear. Here, we found that B cells showed a lymphatic-like distribution in the steady state but a disorganized distribution pattern in human colorectal cancer tissue. These results led us to propose that the change in distribution pattern may affect B cell differentiation and development. Our further study showed that IgG4- and IgA- B cell subsets isolated from normal tissues. More importantly, we also detected higher IgG4 levels in the plasma of colorectal cancer patients compared with healthy donors, suggesting that B cell subsets change in tissues can be reflected in the patient’s body fluids. Evidence for antibody class switching and antibody maturation in human colorectal cancer; support the involvement of B cells in human colorectal cancer immunity.

P.B2.01.15

Serum chemokine profiling reveals candidate biomarkers for recurrence and immune infiltration in ovarian cancer

A. Mlynska1, G. Salucinei2, K. Zillonie1, B. Intaite1, A. Barakauskien3, V. Pasukoniene1

1National Cancer Institute, Vilnius, Lithuania, 2Vilnius University, Vilnius, Lithuania.

The management of advanced ovarian cancer is challenging due to the high frequency of recurrence, often associated with the development of resistance to platinum-based chemotherapy. Molecular analyses revealed the complexity of ovarian cancer with particular emphasis on the immune system that may contribute to disease progression and response to treatment. Chemokines orchestrate the cross-talk between cancer and immune cells, and therefore present as potential biomarkers, reflecting the tumor microenvironment. We examined a panel of circulating CC and CXC chemokines in the serum of 40 high-grade ovarian cancer patients prior to primary surgery. We also analyzed the level of immune infiltration in tumors.The preoperative levels of chemokines differ between patients. Elevated levels of circulating CXCL4+CCL20+CXCL1 combination can discriminate patients with shorter recurrence-free and overall survival. Serum CCL17 has a potential to select platinum-resistant tumors. In half of the patients, we detected the presence of CXCL4+CXCL9+CXCL12+CXCL11 combination. Circulating CXCL4+CCL20+CXCL11 combination in plasma distinguishes immune-infiltrated tumors that are more likely to recur. Our results suggest that profiling of circulating chemokines in ovarian cancer patients may provide valuable information regarding tumor chemosensitivity and immune infiltration. We show that combinations have better prognostic utility than single chemokines and may serve as patient stratification tools.

P.B2.01.16

Diverse functions of CR3 (CD11b/CD18) and CR4 (CD11c/CD18) β2-integrins expressed by human B lymphocytes

Z. Nagy-Bolyai, S. Lukacs1, B. Mácsik-Valent1, Z. Bátyi1, A. Erdé1

1Department of Immunology, Édvard Loránd University, Budapest, Hungary, 2MTA-ELTE Immunology Research Group, Édvard Loránd University, Budapest, Hungary.

CR3 and CR4 are known for long to participate in adhesion and migration of myeloid cells. The expression and function of these β2-integrins on human B lymphocytes however, has not been extensively studied yet. Investigating the CD11b and CD11c expressing human B cell line BJAB we found that blocking CR4 with a CD11c specific antibody, a significant, up to 50 % reduction of adhesion of BJAB B cell subsets change in tissues can be reflected in the patient’s body fluids. Evidence for antibody class switching and antibody maturation in human colorectal cancer; support the involvement of B cells in human colorectal cancer immunity.

P.B2.01.17

Exploring the effects of tetrapspin CD37-deficiency on metabolic signaling during B-cell lymphomagenesis

R. Peeters, A. Hoekstra, R. Steenstra, C. Berkers, E. Jansen, A. Van Spriel

1Department of Tumor Immunology, RIMLS, Radboudumc, Nijmegen, Netherlands, 2Department of Biochemistry and Cell Biology, University of Utrecht, Utrecht, Netherlands, 3Department of Internal Medicine, Radboudumc, Nijmegen, Netherlands.

Introduction: Immune cells employ a metabolic state that fits their specific needs. These needs fluctuate throughout their lifespan. Metabolic alterations are induced by intracellular and extracellular signals that are coordinated by membrane receptors. Abnormal composition of these receptors can have drastic effects on cell. The tetraxspan superfamily of 4-transmembrane proteins controls membrane protein organization. Absence of the immune-specific tetraxspan CD37, results in spontaneous B-cell lymphoma development in mice. Importantly, diffuse large B-cell lymphoma (DLBCL) patients lacking CD37 on tumor cells have a significantly worse prognosis. CD37 controls the activity of Akt kinase which plays a central role in metabolic regulation and cell survival. We therefore set out to establish the effects of CD37-deficiency on the metabolic fate of healthy B-cells and its potential role in lymphomagenesis.

Objective: To provide more insight into the metabolic pathways underlying CD37 function in healthy B cells and during lymphomagenesis, potentially finding modes of clinical intervention.

Methods: Metabolite abundance in WT and CD37-/- of human B-cell lines and primary murine B-cells was assessed with mass spectrometry (MS). The metabolic analyzer Seahorse XF96 was used to directly measure oxidative phosphorylation (OXPHOS) and glycolysis activity in live cells. Furthermore, mitochondrial phenotyping of B-cells was carried out using confocal microscopy.

Results: Preliminary results indicate that B-cells without CD37 display lower metabolic activity. WT cells contain higher absolute levels of OXPHOS, and glycolysis-associated metabolites. Furthermore, WT B-cells portray higher oxygen consumption and extracellular acidification, indicating more active OXPHOS and glycolysis respectively. 
P.B2.01.18
B cells in esophago-gastric adenocarcinoma are highly differentiated, organize in tertiary lymphoid structures and produce tumor-specific antibodies

H. A. Schröfer1, M. Theiler2, A. Lechner3, K. Wenhofel4, B. Gathoff5, R. Gilles6, E. Cukuroglu1, J. Gakele7, A. Quassa8, C. Bruns9, A. H. Hölzer1
1University of Cologne and Center for Molecular Medicine, Cologne, Germany, 2Center for Molecular Medicine Cologne, Cologne, Germany, 3Ludwig Maximilian University Munich, Munich, Germany, 4University of Cologne, Cologne, Germany, 5Gene Institute of Singapore, Singapore, Singapore, 6Genome Institute of Singapore, Singapore, Singapore, 7Ludwig Maximilian University Munich, Munich, Germany, 8German Cancer Consortium (DKTK), Heidelberg, Germany.

Introduction:Tumor-infiltrating lymphocytes (TILs) are correlated to prognosis of several kinds of cancer. Most studies focused on T cells, while the role of tumor-associated B cells (TABs) has especially more attention. TABs contain subpopulations with distinct functions, potentially promoting or inhibiting immune responses. This study provides a detailed analysis of TABs in gastro-esophageal adenocarcinoma (EAC). Methods: Single cell suspensions of tumor samples (n=54), mucosa (n=43), lymph nodes (n=42) and peripheral blood mononuclear cells (PBMC, n=88) of EAC and PBMC of healthy controls (n=20) were studied by flow cytometry. A panel of 34 tumor-associated antigens (TAAs) expressed in EAC was identified based on public databases and TCGA data to analyze tumor-specific B cell responses using a LUMINEX® bead assay and flow cytometry. Spatial distribution of TABs was analyzed by confocal immunofluorescence-microscopy. Results: TABs were elevated in primary tumor samples compared to PBMCs of gastric cancer patients or normal mucosa. Subset-analyses of TILs revealed increased proportions of differentiated and activated B cells and enrichment for follicular T helper cells. TABs were organized in tertiary lymphoid structures (TLS) at the invasive tumor margin. Structural analyses of TLS and the detection of tumor-specific antibodies against one or more TAAs in 48.1% of analyzed serum samples underline presence of anti-tumor immune responses in EAC. B cells were decreased in tumors with expression of Programmed Death Ligand 1 or impaired HLA-I expression. Conclusions: Anti-tumor B cell responses are an additional and underestimated aspect of EAC. These results are of immediate translational relevance to emerging immunotherapies.

P.B2.01.19
Evaluation of serum iron in tumorigenesis and malignancy of ovarian cancer

N. Touns1, A. Benyelles-Boufennara2, B. Ojdjuric3
1University of sciences and technology Houari Boumediene, Algeria, 2Department of Department of Pathological anatomy, Public Health Center Pierre and Marie Curie, Mustapha Basha Hospital (Algeria, Algeria), 3Algeria, Algeria.

Introduction: Ovarian cancer is a lethal malignancy that recorded in 2012, 238719 new cases and caused 151917 deaths, worldwide. It represents the fifth most common women cancer in Algeria, with a worse prognosis for more than 66% of new cases 821 deaths in 2012.

The goal of our study was to evaluate the contribution of serum iron in Algerian ovarian cancer patient’s progression.

Materials and Methods: Hematocytin and eosin (H&E)-stained sections of ovarian tumors were examined for diagnosis (benign or malignant tumor) and staging. Sera from 40 ovarian tumor patients before treatment and 30 controls were sampled and used for seric iron and CA-125 evaluation.

Results: H&E stained sections of ovarian cancer showed that among the 40 patients, 30 are malignant tumor and 10 benign tumor. The 30 malignant tumor displayed 2 patients in stage I, 12 patients in stage II, 11 patients in stage III and 5 patients in stage IV.

Iron levels decreased by 17% (p < 0.05) while CA-125 increased by 98% (p < 0.001) in malignant ovarian cancer patients, compared to healthy individuals. NO significant correlation were shown between seric iron and CA-125 levels (r= 0.2879, p > 0.05). Despite the altered iron levels, it was not significantly associated to tumoral stage progression (r= 0.1265, p > 0.05).

Conclusion: Our preliminary data suggests that seric iron cannot be taken as supportive biomarkers for the diagnosis of ovarian cancer but may partially emphasizes the iron microenvironment enrichment for ovarian cancer initiation.

P.B2.01.20
SERUM AND URINARY LEVELS OF CD222 IN CANCER DISEASES: ORIGIN AND DIAGNOSTIC VALUE

K. Vilkovuš1, E. Petrovičová2, M. Markaš1, A. Drach1, H. Stockinger1, V. Leška1
1Institute of Molecular Biology, Bratislava, Slovakia, 2BSF Sizar s.r.o, Bratislava, Slovakia, 3University Hospital Vienna, Department of Medicine I, Vienna, Austria, 4Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology Medical University in Vienna, Vienna, Austria.

The mannose 6-phosphate/insulin-like growth factor 2 receptor 2 receptor (CD222, M6P/IGF2R) is a multifunctional transmembrane type I receptor, mostly localized intracellularly, less on the surface of all types of mammalian cells. It is known both to transport lysosomal enzymes through their mannose 6-phosphate moieties and to internalize extracellular ligands like insulin-like growth factor 2 or plasminogen. CD222 is involved in regulation of cell proliferation, migration, T cell activation, and apoptosis. Soluble CD222 has been found in various human body fluids. Based on this work, we propose serum soluble CD222 as a general biomarker for tumorigenesis.

Results: H&E stained sections of ovarian cancer showed that among the 40 patients, 30 are malignant tumor and 10 benign tumor. The 30 malignant tumor displayed 2 patients in stage I, 12 patients in stage II, 11 patients in stage III and 5 patients in stage IV.

Iron levels decreased by 17% (p < 0.05) while CA-125 increased by 98% (p < 0.001) in malignant ovarian cancer patients, compared to healthy individuals. NO significant correlation were shown between seric iron and CA-125 levels (r= 0.2879, p > 0.05). Despite the altered iron levels, it was not significantly associated to tumoral stage progression (r= 0.1265, p > 0.05).

Conclusion: Our preliminary data suggests that seric iron cannot be taken as supportive biomarkers for the diagnosis of ovarian cancer but may partially emphasizes the iron microenvironment enrichment for ovarian cancer initiation.

P.B2.01.21
Study of the biological effects of lactoferrin on the prostate cancer cells with varying sensitivity to hormonal therapy

T. Todorovni1, N. Lukancova2, V. Chechun2
1KE Kavetuly Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Kyiv, Ukraine.

Introduction. Correction of prostate cancer (PCA) hormone resistance is one of the top research directions of biology exploration of this localization. One of the perspective approaches is the search of low-toxic substances that increase treatment efficacy.

Aim: to investigate biological effects of lactoferrin (LF) and to find out the possibility of its use to reduce the malignancy degree of human PCA cells by modifying their phenotype.

Materials and Methods: The hormone-sensitive (LNCaP) and hormone-independent (DU-145) human PCA cell lines were cultured with an exogenous LF. The expression levels of ER, PR, Her2/neu, Ki-67, E- and N-cadherin, were monitored by immunohistochemical analysis. The levels of miRNAs were assessed, using q-PCR. The inactive activity of the cells was examined using an usual standard invitro test according to the manufacturer’s instructions.

Results. We established that cultivation of human PCA cell lines with exogenous LF resulted in lowering of steroid hormone receptor expression (ERa and PR). The decrease in the expression of the Ki-67 under the influence of exogenous LF was observed in both cell lines. Also, we established the decrease of invasive activity - by 40% and 30% in DU-145 and LNCaP cell lines, respectively. We found that under the action of exogenous LF there was an increase in the level of expression of oncogetic and oncosuppressive miRNAs in both cell lines.

Conclusions: Thus, we have shown that under the influence of exogenous LF there are changes in phenotypic characteristics and levels of oncogenic and oncosuppressive miRNAs.

P.B2.02.01
Environmental regulation anti-tumor responses - Part 2

P.B2.02.01
Evaluation of local immune response after silencing of IL-10 or IL-10R expression in MC38 tumors

N. Anger, A. Szczygiet, K. Wegierek, J. Mierzewska, M. Napierala, E. Pajtasz-Plasecka, J. Rossowska
L. Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland.

A growing tumor is composed of a variety of cells and factors, which collectively form the tumor microenvironment (TME). One of the cytokines, which is frequently upregulated in cancer is interleukin 10 (IL-10). The majority of reports indicate that IL-10 is a suppressive cytokine with a pro-tumoral effect. However, IL-10 can also enhance the anti-tumor response. The aim of our research was to evaluate the role of IL-10 in MC38 murine colon carcinoma microenvironment through silencing of IL-10 in MC38 cells. Mice with subcutaneously growing MC38 tumors were intratumorally inoculated with lentivectors silencing IL-10 or IL-10R expression (shIL-10 Lvs or shIL-10R Lvs). Characterization of the tumor microenvironment was performed on the 4th and 6th day after LV inoculation. Percentage of tumor infiltrating subpopulations of myeloid and lymphoid cells and their activation stage were evaluated by flow cytometry.

A multimodal immunostaining of CD8 T cells and CD8- and MDSCs was observed in TME on the 4th and 6th days after inoculation with shIL-10 Lvs. However, shIL-10R Lvs and control Lvs induced an antiviral response characterized by high influx of CD8 T cells into tumor on the 6th day after inoculation.

Conclusion: Our preliminary data indicates that Lvs with Lvs induced a stronger antiviral response. This effect seemed to be diminished by reduction of IL-10 in TME. Additionally, the early influx of CD8 T cells observed on the 4th day after inoculation suggests a CTL-dependent antitumor response. This work was financed by National Science Centre, Poland (grant no 2014/15/N/NZ4/04817).
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

250

POSTER PRESENTATIONS

P.B2.02.02 Modeling cancer immunomodulation using epithelial organoid cultures

Y. E. Bar-Eshrai
1, K. Kretzschmar1; P. Araújo1, Z. Sebestyen1, J. Kubal1, H. Clevers1,2,3
1Hubrecht Institute, Utrecht, Netherlands; 2Oncoide Center, Utrecht, Netherlands; 3UMC, Utrecht, Netherlands; 4Princess Maxima Center, Utrecht, Netherlands.

Carcinoid carcinoma (CRC) is one of the most prevalent forms of cancer which develops in a multi-step process from lesions in healthy colon tissue. While mutations in cancerous epithelial cells drive the process of tumorgenesis, interaction of the tumor with the immune system and subsequent evasion from immune-mediated destruction is essential for tumor progression.

Epithelial organoids provide a platform which allows culturing of cancerous and healthy epithelium while retaining tissue-of-origin identity over a prolonged culture period. As such, epithelial organoids are a reliable system to model many biological processes, ranging from normal epithelial differentiation to tumor development. Also, tumor-derived organoids have shown potential to be used in patient-specific drug screens, making a critical step towards personalized medicine. Here, we report a new method to study immune-cancer interactions and assess modulation of the immune response by CRC. We derived organoids from CRC samples and show by transcriptional profiling that organoids maintain differential expression of immune modulatory molecules as seen in primary tumors. Furthermore, we have set up a co-culture system for organoids and T cells to assess immunoreactivity. Indeed, upon co-culture with TCR transgenic CD8+ T cells, organoid killing and cytokine production by T cells was only observed when co-cultured organoids were pulsed with TCR specific native peptides but not with control peptides. In conclusion, our method presented here allows for investigation of immune cell-tumor interaction in vitro and how immunomodulators can be utilized to stimulate tumor eradication. Implementation of this system may thus lead towards new avenues of patient-specific treatment.

P.B2.02.03 CD68+ cells not implicated in Ncf1-mediated tumor progression

M. Y. Bonner, R. Holmdahl;
MIR, Karolinska Institutet, Solna, Sweden.

Cancer is the second leading cause of death globally, according the WHO, with the number of deaths from cancer expected to increase by 70% over the next two decades. The purpose of this study is to advance our understanding in cancer biology and anti-cancer immune response in order to facilitate the development of improved anti-cancer therapies needed to address this concern. Levels of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide are known to increase as premalignant cells evolve into malignant lesions. However, it also play to a role in immune response and communication. The aim of our study is identify which immune cell subtype is able to inhibit tumor growth through NO2X-ROS inhibition. A mutation in the Ncf1, component of the NO2X complex prevents ROS production in B10.Q Ncf1+/− mice. Recent results point to impaired tumor growth in mice with the Ncf1 mutation, in agreement with published studies. Interestingly, when the B10.Q Ncf1+/− mice acquire a functional Ncf1 gene only in CD68+ macrophages, B10.Q Ncf1+/− MN, they also present similarly impaired tumor growth in relation to the wildtype B10.Q mice. We then tested to the potential of CD11c+ cells mediated tumor progression through functional and non-functional Ncf1 molecules. Our results indicate that the potential of CD11c+ lineage involvement in Ncf1 mediated tumor progression in B16F10 melanoma and LLC Lewis lung carcinoma tumor models.

P.B2.02.04 Assessing the role of myeloid cell GCN2 in anti-tumor immune responses

F. Cichon1, J. K. Sonner1, K. Deumelandt1, L. Wolf1, E. Green1, W. Wick1, M. Platten2,3
1German Cancer Research Center, Heidelberg, Germany; 2Department of Neurology and National Center of Tumor Diseases, Heidelberg, Germany; 3Department of Neurology, University Medical Center Mainzheim, Mainzheim, Germany.

Nutrient deprivation is a hallmark of the tumor microenvironment and exerts significant suppressive influence. The tryptophan catabolism has been identified as a central pathway restricting T cell immunity in tumors. In clinical trials inhibitors of the rate-limiting enzyme that mediates tryptophan depletion, indoleamine-2,3-dioxygenase (IDO), further investigations needed to address this concern. Levels of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide are known to increase as premalignant cells evolve into malignant lesions. However, it also play to a role in immune response and communication. The aim of our study is identify which immune cell subtype is able to inhibit tumor growth through NO2X-ROS inhibition. A mutation in the Ncf1, component of the NO2X complex prevents ROS production in B10.Q Ncf1+/− mice. Recent results point to impaired tumor growth in mice with the Ncf1 mutation, in agreement with published studies. Interestingly, when the B10.Q Ncf1+/− mice acquire a functional Ncf1 gene only in CD68+ macrophages, B10.Q Ncf1+/− MN, they also present similarly impaired tumor growth in relation to the wildtype B10.Q mice. We then tested to the potential of CD11c+ cells mediated tumor progression through functional and non-functional Ncf1 molecules. Our results indicate that the potential of CD11c+ lineage involvement in Ncf1 mediated tumor progression in B16F10 melanoma and LLC Lewis lung carcinoma tumor models.

P.B2.02.05 CombImmunotherapy: poly(C) primes glioblastoma for PD-L1 blockade via lymphocyte attraction and activation

J. De Waele1, E. Marcq1, J. Van Audenaerde1, J. Van Loenhout1, C. Deber1, K. Zwaenepeel1, E. Van de Kelft1, D. Van der Planck1, T. Menovsky1, J. Van den Bergh1, Y. Willemsen1, P. Pasquetti1, Z. Bernemann1, J. Lardori1, M. Peeters1, A. Wouters1, E. Smits1
1University of Antwerp, Antwerp, Belgium; 2Antwerp University Hospital, Edegem, Belgium; 3A2 Nikolaos, Sint-Niklaas, Belgium.

Novel therapies are needed to address to abysmal prognosis of glioblastoma patients. Immunotherapy requires combination strategies to unlock its full potential. Here, we investigated the immunomodulatory capacities of poly(C) on glioblastoma cells and its combinatorial potential with programmed death ligand 1 (PD-L1) blockade.

Primary human glioblastoma cells were cultured from residual tumour tissue obtained from standard surgery of glioblastoma patients. Phenotyping was performed using flow cytometry (FCM), immunohistochemistry, qRT-PCR and multiplex electrochemiluminescence. Toll-like receptor 3 (TLR3) signalling was inhibited using chloroquine. Lymphocyte migration was studied using a transwell FCM assay, and activation using FCM and ELISA. Additional PD-L1/PD-L2 blockade was evaluated using ELISA and CSFE-labelled T-cell proliferation.

Poly(C) stimulated a pro-inflammatory secretome by glioblastoma cells, including type I interferons (IFN), interleukin-15, reduced transforming growth factor β, and chemokines CXCL9, CXCL10, CCL4 and CCL5. Concomitantly, PD-L1 and PD-L2 expression on glioblastoma cells was stimulated via TLR3 signalling. Poly(C)-treated glioblastoma cells doubled CD8+ T-cell attraction, and to a lesser extent CD4+ T cells, in part via ligands for CXCR3 and CCR5, while natural killer cell migration was not affected. Lymphocytes co-cultured with poly(C)-treated glioblastoma cells showed enhanced activation (CD69, IFN-γ) and cytotoxic potential (CD107a, granzyme B). Additional blockade of PD-L1, but not PD-L2, further propagated this immune activation.

Our results show that poly(C) triggers glioblastoma cells to secrete cytokines which attract and activate CD8+ T cells, following which blocking of the elevated tumoural PD-L1 further reinforces immune activation. In conclusion, our data proposes poly(C) to strengthen PD-L1 blockade in glioblastoma.

P.B2.02.06 CD55 involvement in the tumor microenvironment

F. Farina1, M. Quintavalle1, M. Locati1
1Università degli Studi di Milano, Milano, Italy; 2Istituto Clinico Humanitas, Rozzano, Italy.

Cyclopin-dependent kinase 5 (CDK5) is a serine/threonine kinase belonging to the CDK family. Several works highlighted CDK5 role in cancer progression and invasiveness both in solid tumors and in hematopoietic malignancies. Until now, no previous work described CDK5 role in Tumor Associated Macrophages. To study CDK5’s involvement in macrophages polarization, we performed both gain- and loss-of-function studies. Preliminary in vitro experiments were performed on THP1 monocytes cell line, differentiated into M0 macrophages. Once differentiated, macrophages polarization was induced after IFNγ (M1) and IL-4 or IL-10 (M2) treatment. Lentinival overexpression of p35 in M0 macrophages was employed as a gain-of-function model, while lentiviral overexpression of CD55-specific shRNA was employed as a loss-of-function model. We observed that CD55 is highly expressed in macrophages at basal level and it is downregulated after inflammatory stimuli. We measured a reduction in CDKS protein expression after M1 phenotype induction (IFNγ), indicating that CDK5 is necessary for a correct M1 polarization. Conversely, p35 overexpressing macrophages showed a decreased pro-inflammatory gene expression. Therefore, these data suggested a clear CDK5 role in TAMs via a p35/CDK5 activation pathway and possible CDK5 involvement in tumor progression. CDK5 silencing, induced a reduction in podosome formation. These data clarify results obtained in vitro and suggest a possible correlation between p35/CDK5 deregulation and cancer invasion. In addition, CDK5 expression in TAMs might be used as prognostic marker for the outcome evaluation of breast cancer patients. We aim to demonstrate that CDK5 inhibition might be a viable anti-metastatic strategy to treat invasive breast cancer.
POSTER PRESENTATIONS

P.B2.02.07
CD15+NK2A+ B cells from Tumor draining lymph nodes correlate with stage in Breast Cancer patients
A. Frazoa, M. Messaoudene, N. Nunez, E. Piazzo, N. Dulphy, A. Toubert, A. Caignard
INSERM, Paris, France.

We characterized the Natural killer (NK) cells that infiltrate tumor draining (TD) lymph nodes (LN), the first site of metastasis of breast cancers (BC). We analyzed by flow cytometry the phenotype of NK cells from TD-LN, (including non-invaded (NI) and metastatic (M)-LN from BC patients) and also NK cells from healthy donor (HD)-LN. First, we show that NK cells from paired NI and M-LN display similar phenotype and M-LN contained low percentages of tumor cells that express ULBP2 and HLA class I Molecules. Compared to HD-LN, TD-LN NK cells highly express NCR, NK2D and NK2GA receptors and characterized by elevated CD62L and CXCR3 expression. TD-LN contained a major compartment of activated CD56brightCD16− NK2A+ and these NK cells are prominent in Stage IIIA BC patients. We found that a subset of LN-NK cells express PD-1. TD-LN NK cells degranulate efficiently after co-culture with BC cell lines. Cytokine activated TD-LN NK cells exerted higher lysis of BC cell lines than HD-LN NK cells and preferentially lysed the HLA class I−PD-L1+ MCF-7 BC cell line. The expression of inhibitory receptor NKIR2D and checkpoint PD-1 by TD-LN NK cells from BC indicate their potential as targets for immunotherapies using anti-NKG2A and/ or anti-PD1 mAbs.

P.B2.02.08
Slan+ monocytes and NK cells contribute to a tumor microenvironment that induces a p21-dependent growth arrest in melanoma cells
F. Fundi1, *, J. Pohl1, A. Cervero2, K. Schädel1
1Dermatology, University Hospital Heidelberg, Heidelberg, Germany, 2Medical Faculty Mannheim, University Heidelberg, Mannheim, Germany.

The cell types of the immune system orchestrate anti-tumor responses and often eliminate malignant cells before primary tumors or metastasis can arise. In the mouse system, it was recently reported that patrolling monocytes initially detect newly formed metastasis and reduce metastatic load by recruiting NK cells. slan+ monocytes (slanMo) represent a subset of human CD14++CD16+ monocytes (homolog of patrolling monocytes in mice) and were previously identified in melanoma metastasis. slanMo are capable of initiating anti-tumor responses based on the secretion of proinflammatory cytokines and the interaction with NK cells is highlighted by an IL-12/IFN-γ dependent positive feedback loop that results in high levels of TNF-α and IFN-γ. Here, we address the question whether the cytokine milieu generated by co-culturing these two cell types influences the growth of melanoma cells and can lead to senescence induction. To this end, we incubated melanoma cell lines with supernatants from slanMo/NK co-cultures. Supernatant treatment resulted in a severely reduced proliferation rate, increased Senescence-associated beta-Galactosidase staining, and a senescence phenotype characterized by strong p21 (CDKN1A) upregulation. This phenotype could be abolished by combined TNF-α and IFN-γ neutralization. We provided evidence that NK cells migrate towards activated slan supernatants in vitro. In addition, we validated the presence of slanMo in melanoma metastasis prior and after immunotherapy, together supporting a mechanism similar to patrolling monocytes in our mouse model. Our data suggests that slanMo are present in melanoma metastasis and contribute to a pro-inflammatory immune microenvironment that inhibits the growth of melanoma cells. Funded by RTGi2099.

P.B2.02.09
NiII) complexes with Mannich bases affect viability and proliferation of rat tumor and non-tumor (bone marrow, macrophages, lymphocytes) cells
M. Glocvchev1, *, E. Zikovska1, B. Andonova-Lilova1, L. Dyakova1, R. Tudose1, E. Mossarova1, O. Costisro1, R. Alexandrarova1
1Institute of Experimental Morphology, Pathology and Anthropology of Science, Sofia, Bulgaria, 2Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 3Institute of Chemistry Timisoara of the Romanian Academy, Timisoara, Romania.

In the present study we have analyzed the influence of four NiI) complexes with ligands containing the antipyrine moiety N,N-bis[-antipyrinylmethyl]-piperazine (BAMP) or N,N′-tetra[-antipyrinyl-1-methyl]-1,2-diaminomethane (TAMEN) on viability and proliferation of cultured rat tumor and non-tumor cells. The following cell cultures were used as model systems in our investigations: permanent cell line [LSR-SF-9R] established from transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin; primary cultures from tumor growths developed after a s.c. implantation of LSR-SF-9R cells (7.5 x 105 cells/animal) in inbred Wistar rats (PRSC) as well as from bone-marrow cells (BMIC), peritoneal macrophages (PM) and spleen lymphocytes (SL) of the same tumor bearing animals. The investigations were performed by MTT test, trypan blue dye exclusion technique, double staining with acridine orange and propidium iodide and colony-forming method. The compounds were applied at concentrations of 10, 50, 100 and 200 µg/ml for 24h, 48h and 72h. The results obtained revealed that: i) NiI)BAMP[(CH3)2CO] and NiI)BAMP[Cl] are more pronounced cytotoxic agents as compared to NiI)TAMEN[(CH3)2CO] and NiI)TAMEN[Cl], for both tumor and non-tumor cells; ii) BAMP are relatively more sensitive to the toxic effects of NiII) complexes as compared to the other used cell culture models; iii) Both ligands (BAMP, TAMEN) do not significantly decrease viability and proliferation of the treated tumor and non-tumor cells. The authors gratefully acknowledge the EU Grant BG05M2OP01- 2.009-0018-01 from 02.06.2017.

P.B2.02.10
TGF beta compromises STING-induced IFN alpha/beta production and tumor regression in spontaneous tumors
M. V. Guérin1, *, F. Regnier2, J. M. Weiss1, V. Feuillet1, L. Vimeux1, M. Thoarue3, G. Renaudel2, E. Donnadieu1, A. Trautmann1, N. Bercovici1
1Cochin Institute INSERM U1016 CNRS UMR8104, Univ Paris Descartes, Paris, France, 2Cochin Institute INSERM U1016 CNRS UMR8104, Univ Paris Descartes, Paris, France, 3Univ Medical Center Freiburg, Freiburg, Germany.

Background: The rate of tumor growth and the responsiveness to therapies depend not only on intrinsic properties of malignant cells but also on the tumor microenvironment. Here, we examined in which conditions targeting the ubiquitous cytokine STING in spontaneous tumors can trigger the production of type I IFN in the tumor microenvironment, inducing tumor regression. Materials and methods: Mice with spontaneous mammary tumors (MMTV-PyMT) received a single intraperitoneal injection of the STING ligand DMXAA. We examined the evolution of tumor growth and performed a molecular analysis of the tumor and immune infiltrate by flow cytometry, fluorescence imaging and transcriptomics. Results: We show that IFNa/β release that conditioned a swift recruitment of neutrophils, followed by a rise in CD8 T cells and monocytes in transplanted tumors. Conclusion: Our results demonstrate that STING ligands promote efficient antitumor immune responses in the mammary tumor model. The data suggest the presence of STING ligands in the tumor microenvironment that induce an IFNα/β dependent growth arrest in melanoma cells. P.B2.02.11
TGFbeta1 polymorphisms may identify gastric adenocarcinoma patients with high risk of metastasis and lower survival rate
I. Lavezres1, A. Gutierrez2, A. Blazquez1, E. Ovejero1, J. Lasa1, A. Lopez1, R. Gomez1, J. M. Martin-Villeneu2
1Universidad Complutense de Madrid, Dpt. of Immunology, Madrid, Spain, 2Hospital Universitario Principe de Asturias, AlcalÌÁ de Henares, Madrid, Spain.

Transforming growth factor β 1 (TGF-β1) is a cytokine involved in the development and malignancy of tumours. Several works attribute a dual effect to the cytokine in the cancer evolution depending on its levels and the stage of the disease. TGFβ1 gene presents several single nucleotide polymorphisms (SNP), related with TGF-β1 levels. We analysed four SNPs (rs1800468, rs1800469, rs1800470, rs1800471) in a group of 78 patients with gastric adenocarcinoma to assess the association between the TGFβ1 and tumor progression. Patients were classified as type I, II, III (non-metastatic) or IV (metastatic), according to their TNM stage. Upon DNA isolation, the polymorphisms were genotyped. PBMC were isolated and stimulated with PMA-Ionomicin and TGF-β1 was measured by ELISA. Survival curves analysis was also performed. rs1800468-G/A genotype was present in 30% of metastatic patients compared to 10.3% of non-metastatic patients (p=0.049, OR=3.17). rs1800469-T/T was absent in metastatic patients, and present in the 19.1% of non-metastatic patients (p<0.03). The combined haplotyped ACTG was present in 15% of the metastatic patients as compared to 3.2% of non-metastatic (p=0.019, OR=7.65). rs1800468-C/C polymorphism yielded a lower expression of TGF-β1 than the C/T or T/T (1.44 and 1.41-fold respectively) variants. Likewise, the rs1800470 T/T polymorphism produced lower TGF-β1 amounts than C/C variant (0.73-fold). Finally, PBMC of rs1800469/T/rs1800470/C bearing patients produce higher TGF-β1 upon stimulation (1.4-fold, p=0.025) and have better survival-rates (85.5%) than rs1800468/C/rs1800470/T patients (28.6%). These polymorphisms may be able to pinpoint metastasis-prone patients, who would need more aggressive therapeutic approaches upon diagnosis.
P.B2.02.12 Impact of photodynamic therapy on the regulation of human immune system in the context of hepatocellular carcinoma
A. Kumar1, O. Morales2, B. Leroux1, C. Frochot1, S. Mardano1, N. Delhez1, E. Boleslawski1.
1Lille Biology Institute, Lille, France, 2INSERM Unit U1189-ONCO THALI, Lille, France, 3ULBP, UMR-CNRS 7274, University of Lorraine, Nancy, France.
Introduction: In photodynamic therapy (PDT), scientists are trying to test the therapy in different cancer models. However, the immunological impact of the therapy is largely unknown. In human-esophageal is the major hallmark for proliferating cancers, hence, we aim to evaluate the impact of 5-Aminolevulinic acid (5-ALA) mediated PDT on human immune cells: HuH7 (p53 over expression), HepG2 (wild type p53) and HepB (partially deleted p53).
Methodology: The expression of Delta-Aminolevulinic Acid Dehydratase (ALAD) and Protoporphyrinogen Oxidase (PPOX) was analyzed through qPCR. The optimal 5-ALA and illumination dosage was determined by treating cells with varying 5-ALA concentration and illumination duration, followed by cellular mitochondrial metabolism analysis.
Thereafter, the cells were treated with these PDT parameters, the ‘conditioned medium’ was recuperated and used to culture cancer cells to analyze the cellular viability and proliferation.
Results: qPCR proves that HCC cell lines express ALAD and PPOX enzymes. Subsequent treatment of the cell lines reveal cancer cell death along with minimal change in proliferation when cultured with PDT treated conditioned media.
Conclusion: The anti-cancer therapy caused cancer cell death in a cell death pattern corresponding to the state of p53, along with an inhibition of cancer cell proliferation suggesting this PDT towards cell-cycle checkpoint regulation. Our preliminary studies have also showed that 5-ALA PDT can induce an immune-regulatory microenvironment, while its capability to prompt an immunogenic cell death and the role of cancer derived exosomes remains to be studied.

P.B2.02.13 Predictive and prognostic role of circulating and tumor-associated NK cells in HER2-positive breast cancer patients treated with neoadjuvant therapy
A. Muntasell1, S. Servitjans, F. Rojí, M. Cabo1, S. Santano1, J. Tusquets1, B. Bermejo1, M. Martinez1, O. Arpi2, M. Martinez-García1, M. Costa-García1, P. Eroles1, I. Vázquez1, L. Serrano1, C. Vilches1, A. Roigard2, A. Lucchini2, J. Albanell3, E. López-Botet4.
1Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain, 2Hospital de la Santa Creu i de Sant Pau, Barcelona, Spain, 3II′ Fundación Jiménez Díaz, Madrid, Spain, 4Institute of Health Research INCLIVA, Valencia, Spain, 5University Pompeu Fabra, Barcelona, Spain, 6Hospital de Sant Pau, Barcelona, Spain, 7Instituto de Investigación Sanitaria Puerta de Hierro, Madrid, Spain.
We investigated the value of distinct NK cell-related variables for predicting pathological complete response (pCR) in primary breast cancer patients undergoing anti-HER2 antibodies (mAbs)-based neoadjuvant treatment. The circulating and tumor-associated NK cells and the CD16A 158VF genotype were analyzed by multiparametric flow cytometry and PCR in a prospective cohort of patients recruited between 2014 and 2016 (n=64). Tumor-infiltrating NK cell numbers were assessed by double immunohistochemistry (CD56+CD3-) in diagnostic tumor biopsies from recruited from 2008 to 2016 (n=139). NK cell-related variables were correlated with pCR adjusted for prognostic factors.
CD16A 158VF genotype was not associated with pCR. Baseline circulating CD57+ NK cells and tumor-infiltrating NK cell numbers respectively showed an inverse and a positive association with pCR (p=0.01 and p=0.001), indicating a role for systemic CD57+ NK cells and of tumor-infiltrating NK cells. It was found that complex in concentration 0.3 mM exhibits higher toxicity towards tumor cells than carboplatin in the same concentration. At the same time, the complex and carboplatin in concentration 0.3 mM have the equal toxicity towards PBMCs. Furthermore, recently, we have demonstrated that cucurbit[7]uril in concentration 1 mM has proper efficacy. Cucurbiturils, macrocyclic cavitands, are promising tools for this purpose.
Herein, we study the effect of carboplatin, cucurbit[7]uril and carboplatin-cucurbit[7]uril complex (1:1) on B16 melanoma cells and on the primary culture of peripheral blood mononuclear cells (PBMCs) of healthy volunteers. The cells were cultivated in RPMI 1640 media with 10% of fetal calf serum in presence of drugs under study in concentration 0-0.3 mM for 48 h (B16) and 72 h (PBMCs). Cytotoxic effect was evaluated by MTT test, proliferative activity was evaluated using CFSE labeling.

P.B2.02.14 The ecto-ATPase CD39 is involved in the acquisition of the immunoregulatory phenotype by M-CSF-macrophages and ovarian cancer tumor-associated macrophages: Regulatory role of IL-27
1INSERM U1232, ANGERS, France, 2INSERM U903, ANGERS, France, 3INSERM U1232, Laboratoire d’Immunologie et d’Allergologie, CHU Angers, ANGERS, France.
Objectives: Tumor-associated macrophages (TAM) are immuno-suppressive cells that can massively accumulate in the tumor microenvironment (ME). In patients with ovarian cancer (OC), their density is associated with poor prognosis. Targeting mediators that control the generation/differentiation of tumor macrophages (Mf) may represent therapeutic challenge to overcome tumor-associated immunosuppression.
Methods: Our laboratory has previously shown that (i) in vitro monocytes treated with M-CSF or GM-CSF induce the generation of M2 and M1 Mφ, respectively and (ii) that in vivo different M-CSF-Mφ are similar to TAM. We analyzed the expression of the membrane ectonucleotidase CD39 in Mφ subsets and its role in the biology of M-CSF-Mφ.
Results: We observed that CD14+ CD163- TAM isolated from ovarian cancer patients and that in vitro generated M-CSF-Mφ express high levels of CD39 compared to M1-type GM-CSF-Mφ. CD39 expressed on TAM and M1 macrophages is able to regulate extracellular ATP into adenosine via ecto-enzymes. We performed gene expression studies in order to determine the presence of high expression of CD39. It was found that complex in concentration 0.3 mM exhibits higher toxicity towards tumor cells than carboplatin in the same concentration. At the same time, the complex and carboplatin in concentration 0.3 mM have the equal toxicity towards PBMCs. Furthermore, recently, we have demonstrated that cucurbit[7]uril in concentration 1 mM has proper toxicity towards either tumor cells or PBMCs. Cucurbit[7]uril can also suppress aCD3-induced PBMCs proliferation.
Conclusions: These data suggest that targeting molecules that maintain the immunosuppressive phenotype of TAM (IL-27 induced CD39, CD161 ligands) could give substantial benefit to the treatment of ovarian cancer.

P.B2.02.15 Carboxiplatin-cucurbituril complex: antitumour activity with reduced immunotoxicity
N. Knauer1, E. Packshina1, E. Kovalenko1, A. Aktanova1, A. Ermakov2, V. Kazlov2.
1Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation, 2Institute of Inorganic Chemistry, Novosibirsk, Russian Federation, 3Novosibirsk State Medical University, Novosibirsk, Russian Federation.
Cancer is the leading cause of mortality worldwide. Platinum(II)-based cytostatic drugs are actively used for antitumour therapy. However they have a plenty of side effects such as immunosuppression because of their toxicity. This problem can be potentially solved by using nanocarriers for drug delivery which allow to reduce systemic toxicity without loss of efficacy. Cucurbiturils, macrocyclic cavitands, can be promising tools for this purpose. Herein, we study the effect of carboxiplatin, cucurbituril[7]uril and carboxiplatin-cucurbituril[7]uril complex (1:1) on B16 melanoma cells and on the primary culture of peripheral blood mononuclear cells (PBMCs) of healthy volunteers. The cells were cultivated in RPMI 1640 media with 10% of fetal calf serum in presence of drugs under study in concentration 0.01-0.3 mM for 48 h (B16) and 72 h (PBMCs). CYTotoxic activity was evaluated by MTT test. Proapoptotic activity was evaluated using CFSE labeling. It was found that complex in concentration 0.3 mM exhibits higher toxicity towards tumor cells than carboplatin in the same concentration. At the same time, the complex and carboplatin in concentration 0.3 mM have the equal toxicity towards PBMCs. Furthermore, recently, we have demonstrated that cucurbituril[7]uril in concentration 1 mM has proper toxicity towards either tumor cells or PBMCs. Cucurbituril[7]uril can also suppress aCD3-induced PBMCs proliferation.
Our findings suggest that cucurbituril[7]uril can be a prospective nanocarrier for decreasing the toxicity of cytostatic drugs which also has its own immunomodulative effects.
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P.B2.02.16 A lipid mediated paracrine signaling network stimulates tumor associated macrophage development in cancer and metastasis
V. Rai1, R. Roy2.
1Institute of Life Sciences, Bhubaneswar-751023 India, Bhubaneswar, India.
Tumor microenvironment consist of dynamic interactions between tumor cells and the surrounding non-transformed cells. Inflammatory cells constitute a major population of the non-transformed cells. Tumor associated macrophages (TAMs), the predominant population of inflammatory cells have major roles in cancer progression and metastasis but the exact stimulus and triggers for the tumor cells-macrophage interaction remains unclear. Autotaxin or lysophospholipaseD (LysPOLD) catalyses the synthesis of lyso phosphatic acid - the smallest phospholipid from lysophosphatidylcholine by its enzymatic action. Autotaxin is implicated in breast cancer, ovarian cancer and many other cancers. Lysophosphatic acid (LPA) is involved in numerous biological processes encompassing cell growth, cell proliferation, cell migration, cancer and metastasis. LPA effects are mediated on different cell types via its cognate G-protein coupled receptors (GPCRs) or non-receptor pathways.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Our recent study has shown that LPA converts monocytes into macrophages both in mice and humans and has an important role to play with immune cells. Here, we show that cancer-associated growth is associated with tumor-associated macrophages via a signature paracrine link. Our studies identify previously unknown signaling link between tumor cells and macrophages. Furthermore, we identify that suppression of this paracrine network can suppress tumor growth. This study suggests that inhibition of this paracrine network may act as a new therapeutic approach to control cancer and metastasis.

P.B.2.02.17
Interest of NK cells to counteract resistance to target therapies in melanoma
L. Rethacker, A. Frazao, M. Avril, A. Coigard; INSERM, Paris, France.

Melanoma incidence is increasing for several decades and metastatic melanoma patients still have a poor prognosis. Since the identification of activating mutations in B-RAF in 50% of melanoma patients treatment with Braf vemurafenib) and then Braf-/-MEK (cobimetinib) inhibtors is the first line treatment for patients bearing a tumor with a BRAF mutation. Despite high response rates of the development of resistance and relapse after a few months is frequent. To find the best combined treatment, we have investigated how resistance to these inhibitors interferes with melanoma cell immunoegenicity to Natural killer (NK) cells. From 3 Braf mutated melanoma cell lines, we have generated vemurafenib resistant variants (R). Paired sensitive (S) and R cells to vemurafenib displayed similar mutational profile, comparable cell growth kinetics and the growth of R variants is maintained in presence of vemurafenib. We found that resistance to Braf is associated to increased immunoegenicity to NK cells. First, NK cell activation (degranulation and IFNy production) is strongly increased in response to R cells. The lysis of R cells by NK cells was significantly increased. Compared to S cells lines, R variants displayed increased expression of NKG2D ligands (MICA, ULBP2), increased Fas, and TRL1RII expression. The acquisition of resistance is associated to increased NK immunoegenicity, increased TRL1 induced apoptosis. These findings outline the interest of counteracting NK cells and NK based immunotherapy for melanoma patients.

P.B.2.02.18
Utilization of high-frequency irreversible electroporation (H-FIRE) to modulate the tumor microenvironment and promote systemic immune system activation in breast cancer
V. M. Ringel-Saladi1, S. L. Coutermarsh-Ott1, R. M. Brack2, K. E. Huei,3 N. B. White4, M. F. Lorenzo,5 R. V. Davolos,5 J. C. Allen1,6
1Graduate Program in Translational Biology, Medicine, and Health, Virginia Tech, Blacksburg, United States, 2Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, United States, 3School of Biomedical Engineering and Sciences, Virginia Tech-Wake Forest University, Blacksburg, United States, 4Department of Biomedical Science, Virginia Tech Carilion School of Medicine, Roanoke, United States.

Introduction: Breast cancer is among the most common malignancies in the US, in 1 in 8 women will develop invasive breast cancer in her lifetime. Despite promising treatments for breast cancer we observe a percentage of metastatic breast cancers that do not respond to treatment. Thus, new therapies to address metastases are direly needed. High-frequency irreversible electroporation (H-FIRE) is a particularly novel and emerging therapeutic approach for tumor ablation. This technique utilizes a series of high-frequency bipolar electric pulses applied via electrodes inserted directly into the tumor to induce cancer cell death. Our overarching hypothesis predicts local treatment of the breast tumor with H-FIRE will stimulate both the innate and adaptive immune system, leading to systemic anticancer response and improved survival.

Materials and Methods: We utilized a mouse 4T1 mammary tumor model and applied H-FIRE to the primary tumor. We evaluated changes in the size of the primary tumor after treatment, as well as metastatic burden and gene expression in the primary tumor at the conclusion of the model.

Results: Here, we show H-FIRE treatment of the primary tumor results in near complete ablation and a shift in the tumor microenvironment from immunosuppressive to pro-inflammatory. Local H-FIRE treatment also significantly reduces 4T1 metastases in animals with an intact immune system, indicating increased engagement of a systemic anti-tumor immune response and improved activation of adaptive immune system.

Conclusions: We anticipate this novel tumor ablation technology will improve conventional treatment strategies and complement emerging immunotherapy approaches targeting primary tumors and metastatic lesions.

P.B.2.02.19
Serum pre-inflammatory cytokines Tnf-a and IL-6 have a strong predictive strength compared to metalloproteases and markers of tumor activity, bone metabolism and cell apoptosis in breast cancer patients with bone metastases.
A. Notopoulos1, A. Sarantopoulou2, P. Notopoulos1, K. Pannas3, C. Iatkaris3, E. Alevroudis3, I. Petrou3, Z. Ionomour3, G. Merioudakis3, E. Zaramplidou3, A. Doumas1, 1Department of Biomedical Science, Virginia Tech Carilion School of Medicine, Roanoke, United States, 2Department of Pharmacology, Medical School of Thessaloniki, Greece, 32nd Propediatric Surgery Department, Aristotle University of Thessaloniki, Thessaloniki, Greece, Greece, 4Department of Nuclear Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Objective: To evaluate the predictive strength of 32 serum markers in breast cancer patients with bone metastases (BC+BM) under treatment.

Methods: The level of all markers has been measured in treatment-naive BC+BM patients (A) at their enrollment in the study, (B) one month later, and (c) after six months. We created a conventional “scan score” based on the size, number and metabolic activity of BM in the initial bone scintigraphy. Levels of p53, bcl-2, TRAIL, caspase-3, Fas, FasL, MIP-1, MIP-2, TIMP-1, DKK-1, OPGL, RANKL, TRAP-5b, BAP and OPN were determined by an ELISA assay, while CEA, CA 15-3, TPA, CA 27.29, CYFRA 21-1, ICTP, P1NP, PINP, PTHrP, IGf1, CT, OC, TNFa and IL-6 were assayed by radioimmunoassay methods. The clinicopathological characteristics and serum markers were compared among the subgroups identified either on the basis of the scan score (A1, A2) or of the development of bone metastases (with a or without). A1 was scored in 35 (52.3%) patients, A2 in 30 (47.7%) patients. Results: A1 subgroup included 107 (60.93%) patients and 46 (30.07%) patients belonged to subgroup A2. IL-6, bcl-2, P53, MIP2 and OPGL/RANKL ratio efficiently reflected the extent and severity of the initial skeletal involvement. TNFa, IL-6, P53, MIP2, and TRAP had the higher predictive strength being significantly lower in all measurements in patients with subsequent disease remission or stabilization.

Conclusion: The preinflammatory cytokines IL-6 and TNFa help to predict more accurately BC+BM subgroups’ clinical behavior.
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P.B2.03.01

HHLA2 (1H7) is highly expressed in hepatocellular carcinoma cells and associated with better patient survival

P. P. Boor,1 K. Sideras,1 K. Biermann,1 J. Verheij,2 B. Takkenberg,3 S. Mancham,4 G. Zhou,5 Q. Pan,5 K. Tran,1 U. Beuers,1 T. M. van Gulik,1 J. N. IJzermans,1 M. J. Brunot,1 K. Zhang,1 D. Sprenger,1 J. Kwekkeboom2,3,4,5
1ErasmusMC, Rotterdam, Netherlands, 2University of Amsterdam, Amsterdam, Netherlands, 3Eijkman Institute for Liver and Intestinal Research, Amsterdam, Netherlands, 4Albert Einstein College of Medicine, New York, United States.

Introduction: HHLA2 is a member of the B7-family and is thought to function predominantly as a T-cell co-inhibitory molecule. We assessed the expression of HHLA2 in hepatocellular carcinoma (HCC) and determined its relation to patient survival. Method: Tissue-microarrays with HCC tumors and tumor-free liver (TFL) tissues were immunohistochemically stained with an antibody against HHLA2 (clone: 566.1) and scored as negative, weak, intermediate, or strong expression. FACS-analysis of single cells isolated from freshly isolated samples was used to further characterize HHLA2 expression. Results: In 27% of patients HHLA2 expression was absent on tumor cells, while 15.5% had weak expression, 37.1% had intermediate expression, and 19.6% had strong expression on tumor cells (n=194). Absent or weak tumor expression of HHLA2 was associated with poorer HCC-specific patient survival compared with intermediate or strong HHLA2 expression (average 72 versus 95 months; p=0.001). HHLA2 expression was predictive of HCC-specific survival independent of baseline clinicopathological characteristics, like liver cirrhosis, alpha-feto protein serum level, tumor size, and number of lesions (HR 0.43; P=0.004). There was no association between HHLA2 expression in TFL tissue and patient survival. FACS analysis showed that HHLA2 was also expressed on CD13+ BDC14+ myeloid dendritic cells and CD14+ cells in tumors. Conclusion: Tumor cell expression of HHLA2 was observed in most HCC patients and is associated with better HCC-specific survival. HHLA2 expression in tumors may be induced in response to immunologic pressure, which may explain the positive association with prolonged survival.

P.B2.03.02

CD5 and CD6 expression levels as prognostic biomarkers for early-stage non-small cell lung cancer

S. Casado-Llombart,1 F. Arandia,1 A. Moreno Manuela,2 C. Salabug Farías,1 A. Herreros Pamares,3 S. Gallach-García,1 I. Simoes,1 E. Carreras,1 M. Consuegra-Fernández,1 A. Blasco,1 A. Conquero Tomás,2 M. Martorell3, E. J. Santos-Lewitense1, C. Camps Herrero1,4, F. Lazaro2,1 R. Sirera1,1
1Neuroreceptors of the Innate and Adaptive System, Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), Barcelona, Spain, 2Laboratorio de Oncología Molecular, Fundación para la Investigación, Hospital General Universitario de Valencia-CIBERONC, Valencia, Spain, 3Departamento de Patología, Universitat de València, Valencia, Spain, 4Servicio de Oncología, Hospital General Universitario de Valencia-CIBERONC, Valencia, Spain, 5Servicio de Anatomía Patológica, Hospital General Universitario de Valencia, Valencia, Spain.

Introduction: The study of the immune surveillance in the tumour microenvironment is leading to the development of new biomarkers and therapies. This research focuses on analysing CD5 and CD6 expression, two lymphocyte surface markers involved in TCR tuning, as potential prognostic biomarkers in resectable stages of Non-Small Cell Lung Cancer (NSCLC).

Materials and Methods: CD5 and CD6 gene expression were analysed by RTqPCR in 201 paired fresh frozen tumour and normal tissue samples of selected NSCLC. The Cancer Genome Atlas (TCGA) database was used to obtain an independent validation patient cohort. Prognostic value was assessed by Cox regression and Kaplan-Meier curves (log rank test), considering significant p < 0.05. Results: Local cohort consisted mainly of men, current or former smokers, with good performance status (PS=0). Patients with higher CD5 expression had significantly increased overall survival (OS, 53.3 vs 9 months, p = 0.011). Multivariate analysis allowed establishment of CD5 expression as an independent prognostic biomarker for OS in early stages of NSCLC (HR=0.59; 95% CI, 0.329-0.883; p=0.014). Further survival analysis of 97 patients from TCGA database, containing gene expression data for normal and tumoural tissue samples, confirmed high expression levels for both CD5 and CD6 as of prognostic value for relapse-free survival (34.84 vs 77.5 months, p=0.023; 35.31 vs 75.5 months, p=0.020, respectively). Conclusions: The present data support CD5 expression level as a novel independent prognostic marker in resectable NSCLC.

P.B2.03.03

Identifying a novel role for fractalkine in T cell accumulation in the visceral adipose tissue of obesity-associated cancer patients.

M. J. Conroy1, A. Melo Rodrigues1, S. Maher1, S. L. Doyle1, E. Foley1, N. Ravi1, J. V. Reynolds1, A. Long1, J. Lysaght1
1Trinity Translational Medicine Institute, Dublin College Dublin, Ireland, 2Dublin Institute of Technology, Dublin, Ireland, 3St. James’s Hospital, Dublin, Ireland.

The global health burden of obesity continues to rise, resulting in increased incidence of associated morbidities. We have previously reported the importance of T cells in obesity-associated inflammation and demonstrated their active migration to the visceral adipose tissue (VAT) of patients with the obesity-associated malignancy, oesophageal adenocarcinoma (OAC). Furthermore, we have reported that chemokine receptor antagonists can significantly reduce such T cell migration to the VAT and may have therapeutic potential to ameliorate pathological inflammation in obesity and obesity-associated cancer. Here, we show that the inflammatory chemokine fractalkine (CX3CL1) is enriched in the VAT of OAC patients. Furthermore, our ex vivo demonstration of fractalkine-driven migration of OAC-derived T cells, suggests that this chemokine plays a role in T cell recruitment to the VAT. In addition, we placed surface expression of the fractalkine receptor CX3CR1, by high expressing circulating CD8+ T cells is endocytosed but not degraded upon encountering fractalkine. We also show that such fractalkine-mediated endocytosis of CX3CR1 is accompanied by enhanced surface expression of ICAM-1 and L-selectin on peripheral blood-derived CD8+ T cells. Interestingly, our analyses identified these molecules in their soluble form among the most prevalent soluble adhesion molecules in the VAT of OAC patients suggesting that an abundance of fractalkine in VAT serves in T cell adhesion as well as T cell recruitment to this tissue in OAC patients. For the first time, these findings identify fractalkine as a potential therapeutic target to release inflammatory and cytotoxic T cells from the VAT and attenuate obesity-associated inflammation in OAC.

P.B2.03.04

Complete tumor desialylation drives tumor growth through hampered CD8+ T cell cytotoxicity

L. A. M. Cornelissen,1 A. Blanas,3 J. C. Van der Horst,2 L. Kruijssen,4 A. Zaal,1 T. O’Toole,2 Y. Van Kooyk1,3,5, S. Van Vliet;1
1VU University Medical Center, Amsterdam, Netherlands.

The tumor microenvironment is immunosuppressive, allowing tumor cells to escape from immune attack. Tumor cells generally display an aberrant glycosylation profile, amongst others characterized by overexpression of sialylated structures. Sialic acids (Sias) are recognized by Siglec receptors, most of which are immune inhibitory receptors. Therefore, we hypothesized that Sias play a crucial role in suppressing anti-tumor immunity. Indeed, we have previously shown that Sias+ melanoma tumor cells, expressing reduced level of Sias on the cell surface, exhibited delayed in vivo growth due to an augmented effector T cell response. However, the role of Sias on other tumor types than melanoma and the effect of complete cancer cell desialylation on the anti-tumor immune response has never been studied. To generate Sia+ colorectal cancer cell line MC38 and desialylated the cells with the use of CRISPR/Cas9. Interestingly, the MC38-Sia- tumor cells displayed enhanced growth in vivo compared to their mock-transfected counterparts. Strikingly, MC38-Sia- tumors contained less CD8+ T cells and these CD8+ T cells had a reduced activation state. In an in vitro tumor killing assay MC38-Sia- tumor cells were less efficiently killed by activated cytotoxic T cells. Together our results indicate that a lack of Sias in colorectal cancer hampers CD8+ T cell cytotoxicity. Introduction: We show for the first time that complete desialylation drives tumor growth, which greatly impacts the design of novel cancer therapies aimed at targeting the tumor glycosylation profile.

P.B2.03.05

DNGR-1 as a Dendritic Cell-Specific Phenotype in Antitumor Immunity

F. J. Cueto,1 C. del Fresno,2 P. Brandi,3 A. Combes,3 A. R. Sánchez-Pauleté,1 M. Enamorado,2 R. Conde-Garrosa,2 I. Meler,4 M. F. Krummel,5 D. Sancho;6
1Institute of Cardiovascular Research for Cardiovascular Research, University of California San Francisco, San Francisco, United States, 2Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), Barcelona, Spain, 3Instituto Nacional de Salud Mental, Madrid, Spain, University of Navarra, Pamplona, Spain.

Classical type 1 dendritic cells (cDCs) are pivotal to antitumor immunity and their infiltration in tumors associates with better prognosis. DNGR-1 is a dead cell-sensing receptor highly restricted to cDC1s, but its role in antitumor immunity has not been clarified yet. Here, we found that DNGR-1 absence did not affect cross-presentation of tumor-associated antigen, tumor growth or responsiveness to anti-PD-1 treatment. However, F3IL-expressing B16 melanoma showed delayed tumor growth in DNGR-1-deficient mice. Indeed, treatment of mice with anti-PD-1, F3IL and DNGR-1-deficient mice led to improved antitumor immunity. Enhanced antitumor immunity in the absence of DNGR-1 was T cell-dependent and correlated with increased infiltration of cDC1s within B16F10 tumors. Absence or blockade of DNGR-1 in the presence of F3IL resulted in increased expression of CCR7 on cDC1s but not on cDC2s. These results correlated with the analysis of data from TCGA datasets with different cancers, which indicates a strong association between the expression of CCR7 genes CCL19 and CCL21 with cDC1 infiltration. Our data show that blockade of DNGR-1 signaling promotes cDC1 infiltration within tumors in the presence of F3IL, suggesting CCR7 upregulation as a potential mechanism.
We show elevated cell survival under oxidative stress. In addition, cells expressing Δ122p53 were resistant to temozolomide treatment and oxidative stress; suggesting Δ133p53β could reduce the sensitivity to temozolomide and promote response to oxidative stress. 10.1 cells expressing a murine ‘mimic’ of Δ133p53 (Δ122p53) were treated with temozolomide or macrophage content, various other immune cell markers and whether the tumors had wild-type or mutant p53. Hypoxic areas in glioblastoma tissue were detected with carbonic anhydrase 9 (CA9) immunohistochemistry and CA9 expression using RNAscope. To determine if Δ133p53β could contribute to the tumour progression and/or promote tumour cell survival in response to oxidative stress, 10.1 cells expressing a murine ‘mimic’ of Δ133p53 (Δ122p53) were treated with tumour-associated fibroblasts (TAFs) and CAFs expressing high levels of CA9. Tumor-associated macrophages (TAMs) and fibroblasts (TAFs) are important components of the tumor microenvironment. This study shows the potential of CA9-targeting approaches to inhibit tumor growth and progression.

We studied the association between CAFs and tumour cell survival in glioblastoma. Tumour cell survival was assessed using colony formation assays. Tumour cell survival was significantly increased in the presence of CAFs. Tumour cell survival was also significantly increased in the presence of CAFs expressing high levels of CA9. Our results suggest that CAFs may contribute to tumour progression and survival by promoting tumour cell survival. This study highlights the potential of CA9-targeting approaches to inhibit tumour growth and progression.

We showed Δ133p53β aids tumor progression by promoting an immunosuppressive and chemoresistant environment. This study suggests a role for hypoxia signalling in the regulation of Δ133p53β expression.
**POSTER PRESENTATIONS**

**PB2.03.11**

**In vivo suppression of murine tumour growth through CD8+ CTL via activated DCs by sequential administration of alpha-galactosylceramide**

H. Kogo1,2, M. Shimizu3, Y. Negishi3, H. Takahashi3
1Department of Microbiology and Immunology, Nippon Medical School, Tokyo, Japan, 2Department of Gastrointestinal and Hepato-Biliary-Pancreatic Surgery, Nippon Medical School, Tokyo, Japan, 3Department of Surgery, Kitamurayama Hospital, Yamagata, Japan.

Tumour immunity is largely attributed the effective priming and activation of tumour-specific class I MHC molecule-restricted CD8+ CTLs. CD205+ DCs can cross-present the epitopes of captured tumour antigens associated with class I MHC molecules alongside co-stimulatory molecules to prime and activate tumour-specific CD8+ CTLs.

Immature DCs (iDCs) with reduced co-stimulatory molecule expression may be a cause of impaired CTL induction. Hepa-1-6 cells were established from the murine hepatoma cell line Hepa-1-6; these cells grow continuously after subcutaneous implantation into syngeneic B6 mice and do not prime CD8+ CTLs. In this research, we show that the growth of ongoing tumours was suppressed by activated CD8+ CTLs with tumour-specific cytotoxicity through the administration of alpha-GaCer, which is a glycolipid known to stimulate iNKT cells and selectively activate CD205+ DCs. Moreover, we demonstrated that subsequent repetitive intraperitoneal inoculation with alpha-GaCer every 48 hours appeared to convert co-stimulatory CD205+ DCs into immunogenic DCs with a higher expression of co-stimulatory molecules and a stronger cross-presentation capacity, which primed CTL precursors and induced tumour-specific CD8+ CTLs within the tumour environment without activating iNKT cells. These findings provide a new method for cancer immunotherapy to convert co-stimulatory CD205+ DCs within tumours through the sequential administration of an immuno-potent lipid/glycolipid, and then activated immunogenic DCs with sufficient expression of co-stimulatory molecules prime and activate tumour-specific CD8+ CTLs within the tumour to suppress tumour growing.

**PB2.03.12**

**Abrogation of the immunosuppressive tumor microenvironment in cholangiocarcinoma by targeting PD-1 or GITR**

Erasmus MC - University Medical Centre, Rotterdam, Netherlands.

Cholangiocarcinoma (CCA) is an aggressive malignancy of the biliary tract. CCA-patients generally present with advanced disease for which no curative treatment is available. Whether CCA is responsive to immune checkpoint antibody therapy is unknown, and little is known about the tumor immune microenvironment of CCA. We characterized tumor-infiltrating lymphocytes (TIL) isolated from freshly resected CCA tumors, determined their expression of co-signaling molecules, and assessed the effects of targeting these molecules on TIL functions in ex vivo assays. TIL contained lower proportions of CD8+ T cells, NKT cells and NK cells and higher proportions of CD4+Foxp3+ regulatory T cells (Treg) than lymphocytes isolated from tumor-free liver tissues (TFL) of the same patients. Immunohistochemistry showed that the majority of CD8+ and CD4+ T cells were sequestered at the tumor margin, while Treg accumulated in the tumors. Tumor-infiltrating CD8+ T cells showed reduced expression of the cytotoxic molecules perforin and granzyme compared to those in TFL and bone marrow.

Co-stimulatory receptor GITR as well as co-inhibitory receptors PD-1 and CTLA4 were over-expressed on tumor-infiltrating T cells compared with T cells in TFL and blood. PD-L1, PD86 and DB80 were expressed on antigen-presenting cells in tumors, but GITR ligand not. Antagonistic targeting of PD-1 with nivolumab or agonistic targeting of GITR with GITR-ligand enhanced granzyne B and effector cytokine production and/or T cell proliferation in ex vivo stimulations of TIL with CD3 and CD28 antibodies. **Conclusions:** The tumor microenvironment in CCA is immunosuppressive. PD-1 and GITR are potentially promising targets for immunotherapy of CCA patients.

**PB2.03.13**

**Bone marrow endothelial cells sustain a tumor-specific CD8+ T cell subset with suppressive function in myeloma patients**

P. Leone, G. Di Lemia, D. Giannico, A. Solimando, A. Vacca, V. Racanelli
University of Bari Medical School, Bari, Italy.

Endothelial cells (EC) line the bone marrow microvasculature and are in close contact with CD8+ T cells that come and go across the permeable capillaries. Because of these intimate interactions, we investigated the capacity of EC to act as antigen-presenting cells (APC) and modulate CD8+ T cell activation and proliferation in bone marrow of patients with multiple myeloma (MM) and monoclonal gamopathy of undetermined significance. We found that EC from MM patients show a phenotype of semi-professional APC given that they express low levels of the co-stimulatory molecule CD40, CD80 and CD86, and of the inducible co-stimulator ligand (ICOSL). In addition, they do not undergo the strong switch from immunomodulation to proteosome subunit expression which is typical of mature professional APC such as dendritic cells. EC can trap and present antigen to CD8+ T cells, stimulating a central memory CD8+ T cell population that expresses Foxp3 and produces high amounts of IFN-γ and TGF-β. Another CD8+ T cell population is stimulated by professional APC, produces IFN-γ, and exerts antitumor activity. Thus, two distinct CD8+ T cell populations exist in the bone marrow of MM patients: the first population is sustained by EC, expresses Foxp3, produces IFN-γ and TGF-β, and exerts pro-tumor activity by negatively regulating the second population. This study adds new insight into the role that EC play in MM biology and describes an additional immune regulatory mechanism that inhibits the development of antitumor immunity and may impair the success of cancer immunotherapy.

**PB2.03.14**

**MESENCHYMAL STROMAL CELL SIALINATION ENHANCES T LYMPHOCYTE IMMUNE SUPPRESSION**

K. Lynch1, M. O’Dwyer1, A. Ryan1, T. Ritter2
1Regenerative Medicine Institute (REMEDi), College of Medicine, Nursing & Health Sciences, National University of Ireland, Galway, Galway, Ireland, 2Department of Pharmacology, College of Medicine, Nursing & Health Sciences, National University of Ireland, Galway, Galway, Ireland.

**Introduction:** Little is known about the mechanisms of immune modulation mediated by mesenchymal stromal cells (MSC) in the tumour micro-environment (TME). Aberrant glycosylation is a hallmark of cancer cells, playing an important role in tumour progression. Here we sought to investigate if regulation of MSC sialylation alters their ability to inhibit T-cell function in an inflammatory micro-environment, characteristic of the TME.**Methods:** MSC were treated with both tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) (1 MSC-1) for 72 hours and the sialic acid levels were analysed by flow cytometry. MSC and i-MSC were co-cultured in MSC-lymphocyte co-cultures for 96 hours. T-cell proliferation, activation, death and differentiation were determined by flow cytometry. Both MSC and i-MSC were pre-treated with a sialyltransferase inhibitor (3FAxNeuSAC) for 72 hours prior to TNF-α and IL-1β stimulation and co-cultured with lymphocytes. **Results:** MSC displayed phenotypical changes. i-MSC displayed increased expression of sialic acid. Both MSC and i-MSC displayed pro-tumor activity in the TME. **Conclusions:** Our findings confirm that inflammation induces MSC sialylation and enhances their ability to suppress activated lymphocytes. We suggest that understanding the importance of MSC sialylation is likely to lead to the identification of a new molecular target.

**PB2.03.15**

**Clinical relevance of intratumoral dendritic cells in neuroblastoma**

O. Meland2,3, M. Chierici2, V. Lucarini3, M. Compagnone3, G. Zicheda1, G. Jurman1, R. Boldrin2, C. Furlanella1, F. Locatelli1, D. Fruci1
1Ospedale Pediatrico Bambino Gesù, Rome, Italy, University of Pisa, Pisa, Italy, 2Fondazione Bruno Kessler, Trento, Italy.

**Introduction:** The prognostic value of tumor-infiltrating T lymphocytes (TIL) has been demonstrated in several human cancers. Recently, we have shown that TILs have a prognostic value greater than, and independent of the criteria currently used to stage neuroblastoma. We defined an immunoscore based on the presence of different T-cell subsets that associates with favorable clinical outcome in MYCN-amplified tumors. We also demonstrated that the combined PD-L1 and HLA class I tumor density represents a novel prognostic biomarker for neuroblastoma. Here we sought to further dissect the neuroblastoma microenvironment, evaluating density of infiltrating dendritic cells (iDC), macrophages and NK cells. Moreover, we tested whether immune gene profiling of neuroblastoma could identify predictive and prognostic targets for these patients.**Methods:** In situ immunohistochemical staining for CD141, CD163 and NKP46 was assessed in 104 neuroblastoma specimens and correlated with clinical outcome. Gene expression profiling using Nanostring nCounter Immune Panel was also performed in 36 neuroblastoma samples. Publically available datasets were used to validate the results. **Results:** High density of iDCs, macrophages and NK cells was correlated with the presence of TILs, tumor HLA class I expression and favorable clinical outcome, suggesting that the presence of these cells correlates with favorable tumour development. These data were confirmed by gene expression profiling analyses. Clustering analysis revealed the existence of distinct expression profiles in high- and low-risk neuroblastomas. **Conclusions:** These results may provide a rationale for improving risk stratification of patients and addressing towards a more targeted therapy.
POSTER PRESENTATIONS

P.B2.03.16 The balance between activated follicular help T-cells and follicular regulatory T-cells infiltrating human breast cancer guides anti-tumor immune responses

G. Noël1, M. Langouët2, S. Garraud1, G. Van den Eynde3, A. Boissier1, H. Daviller1, D. Larmsmont1, K. Williard-Gallo1
1Molecular Immunology Unit-Institut Jules Bordet-ULB, Bruxelles, Belgium, 2Anatomical Pathology Department-GZA Hospital Sint-Augustinus, Antwerp, Belgium, 3Flow Cytometry Core Facility-Institut Jules Bordet-ULB, Bruxelles, Belgium, 'Anatomical Pathology Department-Institut Jules Bordet-ULB, Bruxelles, Belgium.

The recent success of immunotherapy highlights the importance of the immune response in cancer treatment. In human breast cancer (BC), tumor infiltrating lymphocytes (TIL) can organize in tertiary lymphoid structures (TLS) in the stroma. We have shown that CXCL13, a B-cell chemoktractant, is involved in TLS formation and associated with positive clinical outcomes. Therefore, we present a study investigating how T cells functionally behave in TLS in human BC.

We used fresh primary breast tissues to prepare primary tumor supernatants for immunoglobulin analysis and TLS for flow cytometric analysis and sorting. Matched formalin-fixed paraffin-embedded tissues were used for TLS and TIL scoring and organization. CXCR5, the CXCL13 receptor, is expressed on infiltrating B-cells, CD4+ T-cells [follicular helper T (Tfh) cells] and interestingly a CD8+ T-cell subpopulation. All of these CXCR5+ TIL co-localize in TLS, but only ICOSPD-1+ Tfh TIL express high levels of BCL6, IL21, CXCL13 and IFNG mRNA and help B- and helper T-cell differentiation in vitro. ICOSPD-1+ Tfh TLS are also correlated with activated CD8+ TIL in tumors and IgG in tumor supernatants. Follicular regulatory T-cells (Tfr), express GARP, a sign of active TGFβ, within TLS. The Tfh/Tfr ratio is significantly correlated with IgG but also with TGFβ production suggesting that the balance between effector and regulatory Tfh TIL influence TLS activities. Activated ICOSPD-1+ Tfh and GARP+ Tfr TIL are major players in TLS functionality, regulating anti-tumor immune responses and likely playing an important role in the immune response to immunotherapy.

P.B2.03.17 Stromal cell PD-L1 inhibits CD2 T cell anti-tumor immune response and promotes colon cancer

G. O’Malley1, O. Trocy1, K. Lynch1, S. Naicker1, P. Lohan1, P. Dunn1, T. Ritter1, L. J. Egan1, A. E. Ryan1
1National University of Ireland, Galway, Galway, Ireland, 2Queens University Belfast, Belfast, Ireland.

Stromal cells of mesenchymal origin reside below the epithelial compartment and provide structural support in the intestine. These intestinal stromal cells interact with both the epithelial cell compartments as well as infiltrating hematopoietic immune cells, however, little is known about their function and phenotype in the inflammatory tumour microenvironment. Using a syngeneic immunogenic colorectal cancer model, we show that TNF-α initiated inflammatory signalling in CT26 colorectal cancer cells selectively induces PD-L1 expression in stromal cells. Stromal cell PD-L1 potentiates enhanced immunosuppression, characterised by inhibition of T & CD8+ T cell proliferation and, consequently, enhanced tumour progression. To confirm a definitive role for stromal cell PD-L1 in the suppression of T cell proliferation and activation, we targeted the PD-1/PD-L1 signalling axis using a monoclonal blocking antibody to PD-L1. We show that TNF-α initiated inflammatory signalling in CT26 colorectal cancer cells selectively induces PD-L1 expression in stromal cells. We demonstrate that TNF-α induced PD-L1 expression in stromal cells can potentiates enhanced immunosuppression. This study aims to explore the role of TNF-α induced PD-L1 expression in stromal cells in the suppression of T cell proliferation and activation.

P.B2.03.18 The Model Regarding Participation of the Immune System in Metastatic Spread

D. Sepialiashvili
Gynecology Dispensary LTD, Tbilisi, Georgia.

Cancer cells (CC) appearing in a macrophage, may not die because the process of apoptosis is disrupted in them. In the case of "incomplete phagocytosis", concealed in a macrophage CC can move towards the direction of lymphatic capillaries or directly to the blood vessels. The phagocyte with CC freely penetrates into the capillaries, reaches the lymph nodes. CC can multiply in the transport macrophage. After reaching a lymph node, the phagocyte, with incubated CC in it, is dying, inosinisation of CC occurs in the lymph nodes, and the new cycle is put into action.

The sizes of the microorganisms mainly vary within the range 1 - 4 μm. Most zoonoic have the diameter of 10-20 μm. and rarely are changed more than 2 times beyond this range. The sizes of macrophages equal 20-80 μm. Most researchers, considering the sizes of cells, indicate that the stem cells are much smaller in size, than more complete and highly proliferative cells. It turns out, that the sizes of the macrophages and stem cells are of the same kind and comparable. Macrophages are much more actively phagocyte microobjects with a size of 1-4 μm. Perhaps, with small-sized cancer stems, the special "tropism" of macrophages to the latter, is explained. Perhaps, this is the reason for the steady progression of tumor growth.

P.B2.03.19 The effect of radiofrequency ablation on the frequency of CD4+ T cells in patients with inoperable pancreatic cancer

N. Taria1, M. Mizandari2, N. Kikode1, J. Puntusia3, N. Janikashvili1, T. Chikovani1
1Department of Immunology, Tbilisi State Medical University, Tbilisi, Georgia, 2Department of Interventional Radiology, Tbilisi State Medical University, Tbilisi, Georgia, 3Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia.

Introduction: There is an increased interest in radiofrequency ablation (RFA) as the new type of local ablative therapy for inoperable pancreatic cancer. In addition to cancer eradication, RFA favors tumor antigen release followed by the increase in specific anti-tumor immune response. However, this effect lasts for short time period. We suppose that it will be able to maintain anti-tumor immune response with repeatable RFA. The aim of the study was to explore the impact of repeated intraluminal RFA on the frequency of CD4+ T cells in patients with inoperable pancreatic cancer.

Methods: Patients with inoperable pancreatic cancer underwent three repetitive RFA procedures followed by self-expanding stent placement. Peripheral blood was obtained after one month of each procedure. Healthy age-matched volunteers were used as controls. The percentages of CD39+ cells were separately quantified within CD4+ and CD8+ populations. Data were acquired on a FACSArray cytometer and analyzed with FlowJo® v7.5.6 software.

Results: Our results demonstrate that the frequency of total circulating CD4+ lymphocytes was comparable between the patients and controls. After one month of RFA procedure, the frequencies of CD4+ and CD8+ T cells were decreased. The percentage of CD39+ cells was decreased after repeated RFA procedure.

Conclusion: It is the first time to study the effect of repetitive ablation on adaptive immune response and may, therefore, uncover an important new target for therapeutic intervention as well as relevant treatment of this disease.

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P.B2.03.20 The downregulation of PDCD4 induced by progesterone in human endometrial cancer cells

W. Zengto1, X. Wang1,2
1Department of Gynecology and Obstetrics, Clinical Medical School, Jinan, China, 2Department of Immunology, School of Basic Medical Sciences, Shandong University, Jinan, China.

The endometrium is regulated by changing concentrations of ovarian hormones, such as estrogen and progesterone, and shows periodical changes. Apoptosis-related gene programmed cell death 4 (Pdc4) is identified as a tumor suppressor gene that inhibits neoplastic transformation, tumor progression, and translation. It has been reported that multiple factors participate in regulation of PDCD4 mRNA and protein. This study aims to explore the effect and mechanism of estrogen or progesterone on PDCD4 mRNA and protein expression in human endometrial cancer cells. We demonstrated that progesterone could effectively decrease the expression of PDCD4 protein and P38/AKT/mTOR pathway may be involved in the downregulation of PDCD4 protein. In conclusion, these results suggest that the downregulation of PDCD4 induced by progesterone could affect the therapeutic efficacy of progesterone in human endometrial cancer or endometriosis, which may have important implications for progesterone treatment in clinic.

P.B2.03.21 Targeting lysyl oxidase (LOX) favors T cell migration in tumor microenvironment

A. NICOLAS-BOLUDA1, J. Vaquerol1, S. Barrin1, C. Fanturi-Mimoun1, G. Renault1, A. K. Silva2, L. Fouassier1, F. Gazeau1, E. Donnadieu1

In the last decade, there has been an intense development of immunotherapeutic strategies boosting T cells with efficient anti-tumor activities. These include the use of monoclonal antibodies against the immunosuppressive surface molecules such as CTLA-4 and PD-1. However, complete and durable responses are not seen in a fraction of cancer patients. One of the determinants in the success of T cell-based immunotherapies lies on the ability of effector T cells to migrate within the tumor and access its specific antigens. Solid tumors are characterized by an aberrant organization of the extracellular matrix (ECM) in the form of highly reticulated and long linear collagen fibers, which has been shown to affect T cells penetration into tumor islets.
There are currently many strategies in development to target tumor ECM including the inhibition of collagen oxidase, an extracellular copper-dependent enzyme upregulated in many cancers. Here we evaluated the role of the cross-linking enzyme collagen oxidase in a subcutaneous model of human biliary duct carcinoma (EO-1) and dynamic imaging on fresh tissue slices we investigated the consequences of LOX inhibition on the intratumoral migration of T lymphocytes. Our data indicates that LOX inhibition with beta-aminopropionitrile induced a significant decrease in tumor stiffness mapped using shear wave elastography (SWE) that correlates with the increase of the ability of T cell to migrate within the tumor. These experiments support the rationale of combining collagen-degrading strategies with approaches boosting T cells such as anti-PD-1 antibodies.

P.B2.03.22
Novel potential target genes attract the attention on T regulatory cells in malignant mesothelioma
S. Oliveto1, A. Miluzio1, N. Manfrini2, P. Guarini2, S. Curti2, L. Musti1, M. R. Benvenuti2, P. Novelini2, G. Veronesi2, M. Paganin1, S. Biffi2,3;
1InflammFix, Milan, Italy, 2University of Milan, Milan, Italy, 3University of Salford, Manchester, United Kingdom, 4Thoracic Surgery Unit, ASST Spedali Civili, Brescia, Italy, 5Humanitas Clinical and Research Centre, Rozzano, Italy.

Characterization of tumor infiltrating lymphocytes (TILs) is crucial for understanding the mechanisms of cancer progression and immunotherapy reaction. Blockade of immune checkpoints, such as inhibition of CTLA4 and PD-L1, is the master approach to enhance antitumor immunity. Unfortunately, durable responses fail in most patients, suggesting the persistence of immunosuppressive mechanisms. CD4+CD25+Foxp3+ tumor-infiltrating T regulatory cells (Tregs) are responsible for the suppression of effector T cells and, as a consequence, they favour the tumor in escaping the immune defence. Here we show that Mesothelioma tumor infiltrate lymphocytes containing proinflammatory cells. Foxp3+ cells are specifically organized in the infiltrates’ periphery and have extremely high immunosuppressive capabilities. Thus, we isolated intratumoral CD4+ Tregs from surgical resection samples and performed RNA sequencing in order to characterize their molecular signature. Tregs infiltrating MPM upregulated 1) several immune checkpoint genes, such as OX-40, TIGIT and TIM-3, as in other tumors and 2) a specific set of cytokines and chokine receptors. Surprisingly, they expressed also a set of genes never described in Tregs, which underlines the uniqueness of the immunosuppression in MPM. In order to identify the immunosuppression is driven by tumor-derived signals, we are now performing topological profiling of infiltrating Tregs in MPM tumors. Targeting specific Treg cells by manipulating tumor-derived signals may become a novel approach for MPM treatment.

P.B2.04.01
Environmental regulation anti-tumor responses - Part 4
S. Di Marco1, E. Magrini1, K. Berthenet1, C. Garlanda1, C. Garlanda2,1, A. Mantovani2,1;
1Humanitas Clinical and Research Centre, Rozzano, Italy, 2Humanitas University, Rozzano, Italy.

Pro-tumoral role of complement activation in murine sarcoma models
S. Di Marco1, E. Magrini1, C. Perucchini1, K. Berthenet1, M. Barbagallo1, A. Ponзetta1, C. Garlanda2,1, A. Mantovani2,1;
1Humanitas Clinical and Research Centre, Rozzano, Italy, 2Humanitas University, Rozzano, Italy.

Cancer related inflammation (CRI) plays a fundamental role in fuelling tumor appearance and development. Although the important contribution of complement activation to inflammation, its role in CRI still remains understudied. Recently our group demonstrated the pro-malignant role of complement activation in models of mesenchymal (3-MCA-induced) and epithelial (DMBA/TPA-induced) inflammation-driven skin carcinogenesis, showing that mice deficient for the key molecule C3 were protected from tumor development. First we observed the deposition of C3-cleavage products on vessels and tumor cells of sarcomas, while it was absent in normal tissues. C3 deposition on tumor cells was also observed in vitro, both on 3-MCA-derived sarcoma and on different murine cancer cell lines. Interestingly, both in vitro and in vivo experiments suggested that the activation of the classical and lectin pathways were involved in this process. Then, in vivo experiments showed that C3−/− mice were protected from tumor growth in a transplantable model of sarcoma (MN-MCA), as well as in the 3-MCA-induced carcinogenesis model. C3−/− mice showed a protective phenotype which was associated with reduced macrophage and enhanced CD8+ frequencies in tumor, suggesting that these cells could play a role in the protection. Similar results were obtained with C3−/− mice, suggesting that the C3a/C3aR axis was most likely responsible for the protection from tumor development observed in C3−/− mice. Altogether our results indicate that complement activation occurs in tumor and contributes to sarcoma development.
Leptin decreases susceptibility of breast cancer to NK-lysis via PGC-1α pathway: Linking tumor progression with obesity

P.B2.04.05
A. GATT1, Hichem Bouguerra, Amal Gorrob, Stephan Clavel, Jean-François Louet, Guissama Hager
1Faculté des Sciences de Tunis, El Manar, Tunisia, 2University of Tunis El Manar, Faculty of science of Tunis, Tunisia.

Several studies established a link between obesity and breast cancer (BC) development. Yet, the mechanisms underlying this association are not understood. Among the diverse adipocytokine secreted by hypertrophic adipose tissue, leptin is emerging as a key candidate molecule linking obesity and cancer, since it promotes proliferation, migration and invasiveness of tumors. However, the potential implication of leptin on tumor escape mechanisms remains unknown. This study aims to explore the effect of leptin on tumor resistance towards NK and the underlying mechanism. We found that leptin promotes both BC resistance to NK92-mediated lysis and β oxidation on MCF-7, by the up-regulation of a master regulator of mitochondrial biogenesis, the Peroxisome proliferator activated receptor coactivator-1 α (PGC-1α). Using adenoviral approaches, we show that acute elevation of PGC-1α enhances the fatty acid oxidation pathway and decreases the susceptibility of BC to NK92-mediated lysis. Importantly, we identified new regulatory functions of PGC-1α and leptin in regulating the expression of the hypoxia inducible factor-1 alpha (HIF-1α) by tumor cells, a transcriptional factor with pleiotropic role in cancer. We further demonstrate that leptin treatment leads to an up-regulated PGC-1α expression in tumor cells and activates PGC-1α-dependent pathways. Furthermore, tumor cells induce the expression of PGC-1α in NK92 cells suggesting a key role of leptin in the regulation of PGC-1α expression in tumor cells and NK92 cells. Altogether, our results provide preliminary data to open a new therapeutic approach and improve our understanding on the underlying mechanisms linking obesity and breast cancer.

P.B2.04.01
Tumor cells secretome impairs the plasmacytoid dendritic cells compartment in metastatic melanoma

M. Laviron, P. Leyheer, P. Hamon, C. Combadière, A. Baisonnais;
Centre d’Immunologie et des Maladies Infectieuses, Paris, France.

Tumor-associated macrophages (TAMs) are the most abundant cells within the tumor microenvironment and are thought to be involved in tumor growth, metastasis formation and chemoresistance. We recently highlighted the dual origin of TAMs in lung cancer in mice, being composed of both resident interstitial (Res-TAMS) and monocytic-derived (Mod-TAMS) macrophages. These subtypes exhibit different functions, as Mod-TAMS accelerate tumor growth and chemoresistance whereas Res-TAMS are inhibited by chemotherapeutic treatment. Our goal was to investigate the effect of the tumor cells secretome on the plasmacytoid dendritic cells (PDC) compartment in metastatic melanoma. We first showed that melanoma cells secrete components that impair the function of PDCs in vitro and in vivo. Tumor cell supernatants (TCS) impaired cell viability and migration and reduced the production of type-I interferon (IFN-I) and IP-10 by PDCs. In vivo, pretreatment with TCS impaired the survival and anti-tumor activity of PDCs. Finally, we showed that melanoma-secreted factors could up-regulate the expression of the RIG-I ligand LGP2 in PDCs, which activates a signaling pathway leading to the inhibition of type-I IFN production. Our findings indicate that components released by melanoma cells have a relevant effect on the survival and anti-tumor activity of fully differentiated PDCs. The underlying mechanisms identification is underway through the miRNA Seq data analysis. This work was supported by AIRC (IG-15378).

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P.B.02.04.11
Survival and stemness of HT29 colon cancer cells is influenced by self-DNA via crosstalk of TLR9- and autophagy signaling
G. Müzes1, A. L. Kiss2, F. Sápori2
1 2nd Department of Medicine, Budapest, Hungary, 2 Semmelweis University, Budapest, Hungary.

TLR9 and autophagy pathways seem to be bi-directionally involved in carcinogenesis. In cancer cells the biological consequences of the TLR9 and autophagy crosstalking induced by self-DNA is poorly documented. HT29 cells were incubated with genomic(γ), artificially hypermethylated(α), fragmented, and hypermethylated/fragmented(α/γ) self-DNA sequences. Cell viability, induction of apoptosis, cell proliferation, transcriptional alterations of TLR9-signaling and the autophagy process were assayed, respectively. Moreover, autophagy proteins, morphologic features of apoptosis and autophagy, and the presence of colonospheres were examined. Following incubation with γ-, α-, and α/γ-DNAs viability and proliferation of HT29 cells decreased and percentage of apoptotic cells increased, while F-DNA resulted in an appreciably increased cell survival. Methylation of self-DNA resulted in decrease of TLR9 expression, but did not influence the positive effect of DNA fragmentation on overexpression of MyD88 and TRAF6, and downregulation of TNFα. Fragmentation of DNA abrogated the effect of methylation on IRAK2, NFKB and IL-8 mRNA upregulation. G- and F-DNAs significantly upregulated Beclin1, Atg16L1, and LC3 autophagy genes. On a protein level, the results were parallel with the gene expressions. According to TEM, presence of autophagy was observed in each groups. Incubation with m-DNA activated mitophagy, and suppressed tumor cell survival by inducing features of apoptosis. F-DNA treatment enhanced cell survival, activated macroautophagy and lipophagy. As a marker of stemness, CD133+ colonospheres were present only after m-DNA incubation. Our data provide evidence for an interplay between TLR9- and autophagy signaling using HT29 cells subjected to modified self-DNA with remarkable influences on survival and stemness of cancer cells.

P.B.02.04.12
IL 17a, IL 22 and IL 22 serum levels in colorectal cancer
N. Ibrahim1, F. Gander2, C. E. Guldager3, M. M. Omer2
1Background and Aim: T-helper 17 (Th17) pathway plays an important role in promoting colorectal cancer. The aim of this study was to evaluate serum levels of pivotal cytokines (IL-17A, IL-22 and IL-22) and their correlation with clinicopathologic parameters of colorectal cancer. Patients and Methods: 40(19F) patients with colorectal cancer with a median(range) age of 61(30-83) years and 40(18F) healthy controls with no history of any cancer with a median(range) age of 44(25-58) years were included in the study. Preoperative blood samples were collected from the patients. Correlation between serum interleukin levels and stage, differentiation, presence of metastasis, lymph node invasion, perineural and vascular invasions were also evaluated. Results: IL-17, IL-21 and IL-22 levels as pg/ml were 3.1(1.19-3.73), 108.3(11.9-1394), 38.3(38.4-42.9) respectively in patients with colonic cancer. Whereas, they were 1.3(0.7-6.2), 123.12(61.8-1157.6), 21.3(0.15-143.9)pg/ml respectively in healthy controls. IL-17 and IL-22 were found to be increased significantly in patients with colonic cancer (p<0.001). Cut-off values for the significance of IL-17 was found to be 2.755 pg/ml and cut-off value for IL-22 was found to be 35.63 pg/ml. Any patients were found to be correlated with colonic cancer(p>0.05). On the other hand, presence of metastatic lymph nodes, vascular invasion and perineural invasion were all found to be correlated with increased IL-17 and IL-22 levels(p<0.001). Patients with distant metastasis(n=5) also had significantly increased levels of IL-17 and IL-22. Conclusion: There was a strong correlation between increased levels of IL-17 and IL-22 not only with the presence of cancer but also with the presence of metastasis and differentiation.

P.B.02.04.13
The sweet side of pancreatic cancer: the tumor glyco-code contribute to the tolerogenic microenvironment
E. Rodriguez1, S. Schetters2, G. Kazemier3, E. Giovannetti4, E. Martínez-Viñambres2, L. Villar2, E. Roldán2
1Department of Molecular Cell Biology and Immunology, ULMc., Amsterdam, Netherlands, 2Department of Surgery, ULMc., Amsterdam, Netherlands, 3Department of Medical Oncology, CCA, ULMc., Amsterdam, Netherlands.

Pancreatic cancer, one of the most aggressive malignancies, is characterized by an immune-suppressive tumor microenvironment. It has been postulated that changes in the glycosylation of tumor cells, which we call tumor glyco-code, have an impact in the induction of a tolerogenic microenvironment. Immune cells express different glycan-binding receptors (GBR) that can sense and respond to changes in the glyco-code; which often leads to inhibitory immune processes. In this work, we characterize changes in glycosylation during pancreatic cancer progression and analyze how they interact with the immune system.

An extensive glycoprofiling analysis in 10 different pancreatic cancer cell lines, which revealed that fucosylated antigens (eg. Lewis x, Lewis y, VIM-2) are expressed in cells that markers an epithelial phenotype, while they are absent in mesenchymal-like cells. The presence of those structures is associated with the expression the enzyme GALNT3, which expression is regulated by E2F1 during epithelial to mesenchymal transition.

Interestingly, tumor cell lines are capable to induce the differentiation of monocytes towards DC-SIGN+ tumor associated macrophages (TAMs), characterized by the expression of CD163, CD14 and the mannose receptor. This data suggests that tumor-induced DC-SIGN+ TAMs cells could be differentially modulated by epithelial or mesenchymal cells. DC-SIGN signalling in TAMs may be a characteristic in their interaction with epithelial cells and therefore contribute to early local tolerance in the primary tumor.

P.B.02.04.14
Refractory activation state of CD29 in multiple myeloma plasma cells from extramedullary sites as a possible new mechanism to explain their disseminated behaviour
A. Roncancio-Clavijo1, E. Martínez-Viñambres2, L. Villar2, E. Roldán2
1Hospital Universitario Ramón y Cajal, Madrid, Spain.

Multiple MM (m) is a disease characterized by malignant proliferation of clonal plasma cells (PC), usually restricted to the bone marrow (BM). However, in a subset of cases clonal PC expansion can occur outside of the BM and can present as plasma cell leukemia (PCL) or extramedullary MM (EMM). The mechanisms that explain PC egress out of the BM are poorly understood. Here we report that, in contrast to MM cases without disseminated disease, malignant PC in blood or extramedullary sites did not express the high-affinity form of CD29 and were refractory to cation-induced CD29 activation. Patients and methods: Six MM patients with EMM or PCL and 15 with disease confined to the BM were studied. BM aspirates, peripheral blood or pleural effusions were stained with conjugated mAb against CD38, CD138, CD29 (clone MAR4 or HUTS21, detecting constitutive or active epitopes, respectively). For HUTS21 regulation experiments, tumoral cells were incubated with different Mn2+ concentrations (0.01-10mM). Antigen expression was monitored by flow cytometry. Results: PC from EMM or PCL patients displayed very low levels of HUTS21 active epitope (median: 1.8%±1.6%), in contrast to PC from MM patients with disease confined to the BM (median: 58%±17% p<0.001). Moreover, clonal PC in pleural fluids or blood showed a very poor response to exogenously added Mn2+, even at 10mM concentration (median: 2.6%±5.2%) in comparison to PC from patients without disseminated disease (median: 93%±5%; p<0.001). Conclusion Malignant PC from EMM or PCL patients did not express active CD29 since were unresponsive to regulatory factors.

P.B.02.04.15
The p38 MAPK pathway influence of dendritic cell-treated by tumor-cell-soluble factors
A. S. A. Santos, E. M. Araújo, A. M. Vale
Federal University of Maranhão, São Luís, Brazil.

Introduction: Dendritic cells are antigen-presenting cells responsible for initiating adaptive immune response or induce peripheral tolerance. The microenvironment can induce change in DCs functional status. Thus, in tumor microenvironment, the suppressor factors cause tonal status. Thymic DCs that contribute to the development and progression of cancer. The aim was evaluated the DCs differentiation from human monocytes (Mo-DCLs) in presence tumor-derived soluble factors, as well as a possible signaling pathway involved in this process. Materials and Methods: Mo-DCLs were obtained from monocyte culture with GM-CSF and IL-4 and TNF as maturation stimulus. During the differentiation process, DCs were treated with p38 MAPK pathway inhibitor and 30% (v/v) supernatant of tumor cells MCF-7 and analyzed morphologically and phenotypically. Results: The results showed that the protocol used was efficient for the generation of DCs in vitro. Supernatant did not alter tumor HLA-DR expression, however, reduced the expression of costimulatory molecule CD86. The combined treatment with tumor supernatant and p38 pathway inhibitor promoted an increase in HLA-DR expression, indicating that p38 pathway may be involved in DC differentiation process. However, DCs treated tumor supernatant had less CD86 expression continued with this profile even with the use of a p38 inhibitor, indicating that the p38 pathway may not be the only one involved in this process. Conclusions: Thus, the results suggest that the supernatant of tumor cells alters the functional characteristics of DCs, acting in the maturation process, and that this influence of the supernatant can be dependent upon, but not exclusively, the p38 pathway.
Monocytic myeloid-derived suppressor cells (M-MDSCs) are major regulators of immune responses in cancer as greatly evidenced in melanoma patients. Clinical studies have shown that circulating MDSC levels are closely related to tumor stage and prognosis. This tumor-induced conversion of monocytes into M-MDSCs promotes cancer progression by dampening spontaneous and therapeutic immune responses. A better understanding of M-MDSCs conversion and suppressive function is needed to identify potential targets for new therapeutic approaches targeting this cytokine.

We showed that OSM was overexpressed in human cSCC as well as other cytokines such as IL-6, IL-1β, IFNγ whereas IL-4 was decreased, suggesting a Th1/M1 polarization of cSCC microenvironment. In vitro, OSM induced STAT-3 and ERK signalization, modified gene expression, promoted proliferation and migration of malignant keratinocyte PDVC57 cells. OSM induced STAT-3 and ERK signalization, modified gene expression, promoted proliferation and migration of malignant keratinocyte PDVC57 cells. OSM induced STAT-3 and ERK signalization, modified gene expression, promoted proliferation and migration of malignant keratinocyte PDVC57 cells.

Collectively, these results support a pro-tumoral role of OSM in cSCC development and suggest a new therapeutic approach targeting this cytokine.
POSTER PRESENTATIONS

P.B2.05 Environmental regulation anti-tumor responses - Part 5

P.B2.05.01
Myeloid derived suppressor cells in smokers and in chronic obstructive pulmonary disease patients with and without COPD

V. Andreu1,2, V. Cinelli1, J. Verdú1, Capó-Serra2, M. I. Landi1, A. Iglesias1, A. Alonso3, B. G. Cosío1,3, J. M. Ferrer1,3, J. Saulé3, J. Pons1,2;
1Immunology department. Hospital Universitari Son Espases, Palma De Mallorca, Islas Baleares, Spain, Palma, Spain;
2Institut d’Investigació Illes Balears (IdISBa), Palma, Spain;
3Hospital Universitario Son Espases, Mallorca, Balearic Islands, Spain, Palma, Spain;
4Neumology department. Hospital Universitari Son Espases, Palma De Mallorca, Islas Baleares, Spain, Palma, Spain.

Introduction: Chronic obstructive pulmonary disease (COPD) and lung cancer (LC) are prevalent diseases causing morbidity and mortality worldwide. Tobacco smoking is the main risk factor for both diseases. However, not all smokers develop COPD or LC. Several inflammatory cells have been involved in both diseases and can favor the chronic inflammatory process and/or promote carcinogenesis. Myeloid derived suppressor cells (MDSC) contribute to maintain the anti-cancer and the homestasis of the inflammatory process.

Objective: We aimed to evaluate MDSC subpopulation in COPD and LC patient.

Materials and methods: Percentages of MDSC (Lin2 DR1 -D1CD3+) were evaluated by flow cytometry in peripheral blood samples from COPD and smokers. In both groups, patients with and without LC were also separately evaluated.

Results: Although we did not find differences between COPD patients and smokers without COPD, we observed a trend toward higher percentages in the latter group (3.83±0.83 and 5.03±2.00 respectively; p<0.52). Percentages were higher in COPD patients with LC compared to COPD patients without LC (6.26±0.92 and 1.02±0.58% respectively; p<0.19) however the differences did not reach statistical significance again. Similarly, there was a trend toward higher percentages of MDSC in smokers with LC compared to smokers without LC (6.72±3.31 and 2.61±0.06% respectively; p=0.34).

Conclusion: We have found a trend toward higher percentages of MDSC in smokers without COPD when compared to COPD patients. When we evaluated LC patients in COPD and in smokers without COPD there was a trend toward higher percentages of MDSC in LC patients from both groups.

P.B2.05.02
Evaluation of soluble HLA-G and plasma cytokines in papillary thyroid carcinoma patients

B. C. Berta1, J. N. de Araújo1, J. Sadasiv2, P. Sonet3, F. C. Dias1, R. H. Bortolini1, N. L. de Figueiredo-Feitosa1, L. C. de Freitas1, S. R. Tarrap1, C. C. Ramos1, A. D. Luchessi2, J. C. Freitas2, L. M. Maciel1, V. N. Silbiger1, E. A. Donad3;
1University of São Paulo, Ribeirão Preto, Brazil;
2Federal University of Rio Grande do Norte, Natal, Brazil;
3Hospital Liga Norte RioGrandense Contra a Câncer, Natal, Brazil.

Introduction: Considering that inflammation is a major component of papillary thyroid carcinoma (PTC) pathogenesis, we investigated the role of the immune checkpoint soluble HLA-G molecules and a panel of plasma cytokines in PTC patients. Materials and Methods: We studied 85 PTC patients before thyroidectomy and 80 healthy controls. Plasma levels of sHLA-G and 13 different cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IFN-α, IFN-γ, TGF-β1 and TNF) were determined by ELISA and cytometry, respectively. Data were evaluated using univariate and multiple regression analysis, and ROC curves. Results: Compared to controls, IL-6 levels were increased, while IL-1β, IFN-α and TGF-β1 levels were decreased in PTC patients. IFN-α and TGF-β1 efficiently discriminated patients from controls after multiple logistic regression analysis, in which IL-1β and IL-12p70 levels were the best independently associated with larger tumors (>2.0 cm), while decreased sHLA-G levels were associated with local invasion of the tumor. PTC patients exhibiting poor therapy response presented higher levels of IL-5 and IFN-α. Conclusions: The decreased sHLA-G plasma levels observed in patients do not reflect the increased expression of HLA-G reported for PTC samples. The IFN-α and TGF-β1 levels were able to discriminate patients from controls. Additionally, pro-inflammatory and anti-inflammatory cytokines were associated with poor prognostic factors of PTC and poor response to therapy. Financial support: São Paulo State Research Foundation (FAPESP-grant #2015/26556-0), Federal Brazilian Research Foundations (CNPq-grants #804931/2014-4) and CAPES (CAPES/PROCAD-grant #88881-068436/2014-01).

P.B2.05.03
Investigation of the role of IL-36 cytokines in colon cancer

E. Brint1, A. Houston, C. O’Donnell1;
1University College Cork, Cork, Ireland.

The IL-36 cytokines (IL-36α, IL-36β and IL-36δ) are a recently described subset of the interleukin-1 (IL-1) family of cytokines. Given the involvement of other IL-1 family members in the tumorigenic process, it is highly likely that these novel IL-36 cytokines also play a role in cancer. Here we show that IL-36α and IL-36δ is increased in human colorectal cancer tissue compared to adjacent non-tumour tissue, at both the mRNA and protein, whilst IL-36β is altered at the mRNA level only. Expression did not, however, correlate with stage, grade or patient prognosis. Transcription of the IL-36α receptor (IL-36R) was unchanged, with both tumour cells and immune cells in the tumour microenvironment expressing the receptor. Whilst no IL-36 cytokine altered colon cancer cell migration or invasion, IL-36δ strongly increased cellular proliferation in two colon cancer cell lines. IL-36α and IL-36δ also induced high levels of expression of CXCL-1, CCL-2, CCL-20 and IL-8 in colon cancer cells. Finally, a study of all IL-36 cytokines is strongly induced in these cells in response to the colonic tumour-promoting stimulus PGE2. Taken together, these data show that certain IL-36 cytokines are increased in colon cancer and that tumour cells may respond to IL-36R stimulation in terms of a change in cell proliferation and an induction of pro-tumorigenic chemokines.

P.B2.05.04
Cross-presentation of tumour-associated antigens by lung DC1 is lost in tumours by downregulation of TIM4 mediated effoeryosynthesis

N. Caronni1,2, F. Simoncelli1, G. M. Piperno1, K. E. Cervantes Luevano1, B. G. Cosío1,2, J. Pons1,2, L. C. de Freitas1, S. Bicciato2, F. Benvenuti1;
1Icgeb, Trieste, Italy;
2Center for Genome Research Dept. of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.

Batf3-dependent DC1 are critical for the initiation of anti-tumor immunity and their abundance in tumors correlates with good prognosis and responses to immunotherapies. Yet, the mechanisms by which lung DC1 can effectively cross-present tumor antigens at early stages whereas this activity is lost in established tumors. DC1 isolated from tumor bearing NSCLC. DC1 effectively cross-presented tumor antigens at early stages whereas this activity was lost in established tumors. DC1 isolated from tumor bearing lungs and fed with antigen ex vivo confirmed a selective loss in trafficking of MHC class-i immune complexes from ingested antigens. Gene expression profiling showed upregulation of transcripts involved in antigen processing/intracellular trafficking and exhaustion of inflammatory pathways. Notably, tumor associated DC1 strongly down-regulated Tim4, a receptor for phosphatidylserine implicated in apoptotic cell uptake (efferocytosis) in macrophages. We found that Tim4 expression is restricted to DC1 among lung phagocytes and we confirmed expression of cell proliferation by flow cytometry in tumor associated DC1. Notably, the uptake of dying cells and the cross-presentation of cell-associated antigens was significantly diminished in tumors. In addition, TIM4 blockade inhibited efferocytosis by DC1 and cross-priming of tumor specific T cells. These data identify TIM4-mediated engulfment of dying cancer cells by DC1 as a lung specific mechanism of immune surveillance that is lost upon tumor progression.

P.B2.05.05
Modulation of Glucocorticoid Induced Tumor Necrosis Factor Receptor (GITR)-GITLigand (GITR) interaction in Breast Cancer Cells under the control of Ataxia Telangiectasia Mutated (ATM) Promotore

B. Dayane1, D. Yonen Ernini1, E. Dayane1, G. Esenagi2;
1Izmir Biomedicine and Genome Center, Izmir, Turkey;
2Hacettepe University Cancer Institute, Ankara, Turkey;
3Izmir University of Economics, Faculty of Medicine, Izmir, Turkey.

Despite treatment, breast-like cancers (BLBC) has poor prognosis and high mortality. We investigated ATM activity in BLBC cell lines with radiation and examined the viability of BLBC cells during the GITR-GITRL interaction. ATM expression levels in basal-like (MDA-MB-231, HCC38, MDA-MB-468) and luminal (MCF-7, BT-474, SK-BR-3) breast cancer cell lines were found similar. We observed an increase in ATM (S1981) phosphorylation in these cells. HCC38 cells transfected with “pATM-GL” Luciferase reporter plasmid showed high basal and post-radiation ATM activity with experiments suggesting a post-transcriptional control mechanism. When we investigated GITR and GITL expression in BLBC cells, we observed no change in expression levels with radiation. While MDA-MB-231 and MDA-MB-468 cell lines show high GITRL expression, HCC38 cell line was GITR positive. GITR+ HCC38 cells were incubated with recombinant GITL protein at different serum concentrations (1% and 10%) and the change of cancer cells’ viability, proliferation and amount of membrane-activable cells were investigated with DRAQ7 staining, CFSE assay and MIT assay, respectively. Even though GITR stimulation only has not changed viability and proliferation of HCC38 cells, both ionizing radiation and GITR stimulation had a cumulative effect on cell viability. When cell death was assessed, a significant decrease in viability of the cells was observed, with simultaneous exposure to 80 ng/ml GITRL and 5 Gy ionizing radiation. This study demonstrated that cumulative effect of GITR stimulation and ionizing radiation may affect the viability of breast cancer cells.
POSTER PRESENTATIONS

P.B2.05.06
Glioblastoma exploits glycosylation-mediated immune regulatory circuits for immune escape

S. A. Dusarova1, J. Verheij2, E. R. Abels1, E. C. Rodrigues1, J. S. Van Vliet1, D. P. Noske1, J. Würdinger1, X. O. Braakfeldt1, M. L. Broekman1, Y. Van Kooyk1, J. J. Garcia-Vallejo2
1VU University Medical Center, Amsterdam, Netherlands, 2Massachusetts General Hospital, Harvard Medical School, Boston, United States, 3University Medical Center Utrecht, Utrecht, Netherlands.

Glioblastoma (GBM) is the most aggressive brain malignancy. Its histopathology is characterized by a significant infiltration with tumor associated macrophages and microglia (TAM), often comprising more than 30% of the tumor mass. TAMs express the macrophage galactose lectin (MGL) receptor in several tumor types, where it is thought to contribute to immune suppression upon binding to truncated O-linked glycans. Here we aim to elucidate the role of truncated O-glycans in GBM immune escape. To this end, GBM and control surgical samples were collected, and expression of MGL and its ligands was measured by flow cytometry, immunofluorescence, and ELISA. We detected significantly higher levels of the MGL receptor, and MGL-ligands in patient-derived GBM samples as compared to control samples. We then investigated the in vivo effects of varying levels of MGL ligands on immune composition in the tumor as well as systemically using an orthotopic immunocompetent GBM mouse model. Our data shows increased expression of MGL within several subpopulations of myeloid cells in comparison to mock injected mice. Our high dimensional intratumoral cytometry analysis of tumors overexpressing truncated O-linked glycans revealed significantly increased subpopulations of immune suppressive TAMs. Our results suggest that GBM overexpress truncated O-linked glycans, and exploits glycosylation-mediated immune regulatory circuits for immune escape via immune suppressive TAMs. We hypothesize that manipulation of the MGL-MGL-ligand axis may provide new therapeutic avenues in preventing GBM immune escape.

P.B2.05.07
IDO dependent attenuation of NK cells contributes to enhanced malignant tumor growth in diabetic mice

N. M. Gajovic, M. Jenistevic, N. Arsenijevic, M. Lukic, I. Jovanovic
1Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia, 2Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Serbia, Kragujevac, Serbia.

Diabetic patients have higher incidence and mortality of cancer. Recent study revealed that hyperglycemia-induced oxidative stress is involved in the acceleration of tumor metastasis. We used model of high dose streptozotocin-induced diabetes to investigate its effect on tumor growth and modulation of antitumor immune response of 4T1 murine breast cancer in BALB/c mice. Diabetes accelerated tumor appearance, growth and weight, which was associated with decreased NK cells cytotoxicity against 4T1 tumor cells in vitro. Diabetes induced increased frequencies of systemic NGK2D, perforin, granzyme, IFN-γ and IL-17+ NK cells, while increased level of PD-1 expression and production of IL-10 in NK cells. Diabetes decreased percentage of NGK2D+ NK cells and increased percentage of PD-1+ NK cells also in primary tumor. Diabetes increased accumulation of IL-10+ Tregs and TGF-β1 mediated suppressor cells (MDSCs) in spleen and tumor. Diabetic sera in vitro significantly increased percentage of KLRG-1+ and PD-1+ NK cells, decreased percentage of IFN-γ+ NK cells, expression of NKp46 and production of perforin, granzyme, CD107a and IL-17+ per NK cell in percent to glucose added mouse sera and control sera. Significantly increased percentages of inducible nitric oxide synthase (iNOS) and indoleamine 2,3-dioxygenase (IDO) producing MDSCs and dendritic cells (DC) were found in the spleens of diabetic mice prior to tumor induction. 1-methyl- DL-trypophan, specific IDO inhibitor, almost completely restored phenotype of NK cells cultivated in diabetic sera. These findings indicate that diabetes promotes breast cancer growth at least in part through increased accumulation of immunosuppressive cells and IDO mediated attenuation of NK cells.

P.B2.05.08
IL-20 promoted tumor growth in hepatocellular carcinoma

Y. H. Hsu3, M. S. Chang2
1Institute of Immunology, 69120-Heidelberg, Germany, 2Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia, 3Department of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

Introduction: IL-20 is a proinflammatory cytokine involved in rheumatoid arthritis, atherosclerosis, and osteoporosis. However, the role of IL-20 in hepatocellular carcinoma (HCC) is unclear. We explored the function of IL-20 in HCC Materials and Methods: Tumor tissue samples were analyzed the expression of IL-20 and cyclin D1 by using immunohistochemistry staining and quantitative real-time polymerase chain reaction analysis. To examine the role of anti-IL-20 monoclonal antibody in tumor growth, BALB/c mice was injected with ML-1 cells and treated with anti-IL-20 monoclonal antibody. Results: HCC tumor tissue expressed higher levels of IL-20 than did non-tumor tissue. High IL-20 expression in HCC was correlated with poor overall survival. IL-20 and cyclin D1 expression were also highly correlated in HCC patient specimens and 3 human HCC cell lines. IL-20 also increased cell proliferation and migration, and it regulated matrix metalloproteinase (MMP)-13, tumor necrosis factor (TNF)-α, cyclin D1, and PI3K/Akt in expression in MCL-1 cells. Anti-IL-20 monoclonal antibody attenuated tumor growth in mice inoculated with ML-1 cells. The expression of cyclin D1, TNF-α, MMP-9, and vascular endothelial growth factor (VEGF) was significantly inhibited after anti-IL-20 monoclonal antibody treatment. Conclusions: IL-20 plays a role in the tumor progression of HCC. IL-20 might be a useful predictive marker for HCC progression.

P.B2.05.09
ACKR2 in hepatocellular carcinoma as a checkpoint for neutrophil recruitment and -metastatic activity

M. Massara1, O. Bonavitac1, B. Savino1, N. Caronni1, V. Mollica Poeta1, M. Sironi1, E. Setteni1, C. Recordati1, L. Croci1, F. Picara1, A. Mantovani1, M. Locati1, B. Ronechini1
1Humanitas Research Hospital, Rozzano, Italy, 2University of Milan, Milan, Italy, 3University of Milan, Milan, Italy.

1H. 96 Normal 0 14 false false false IT X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table normal"; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Calibri; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-fareast-font-family:Times New Roman; mso-fareast-theme-font:minor-fareast; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-ansi-language:en-US;} Atypical chemokine receptors (ACKRs) are regulators of neutrophil trafficking, inflammation, and immunity. ACKR2 is a scavenger for most inflammatory CC chemokines and is a negative regulator of inflammation. We reported that ACKR2 is expressed in hepatopoietic precursors and downregulated during myeloid differentiation. Genetic inactivation of ACKR2 results in increased levels of inflammatory chemokine receptors and release from the bone marrow of neutrophils with increased anti-metastatic activity. In a model of Neut1-driven primary carcinogenesis ACKR2 deficiency is associated with increased primary tumor growth and protection against metastasis. ACKR2 deficiency results in neutrophil-mediated protection against metastasis in mice orthotopically transplanted with 4T1 mammary carcinoma and intravenously injected with B16F10 melanoma cell lines. Thus, ACKR2 is a key regulator (checkpoint) of mouse myeloid differentiation and function and its targeting unleashes the anti-metastatic activity of neutrophils in mice.

P.B2.05.10
Factor H (FH) binding and elevated expression levels of membrane complement regulators (mCRP) are key players of enhanced complement resistance of breast cancer cells upon treatment with Paclitaxel and Doxorubicin

M. H. Nashes1, M. Kirschfink1,2
1Institute of Immunology, 69120-Heidelberg, Germany, 2National Research Center (NRC), Cairo, Egypt.

Tumor resistance to chemotherapy is a major problem in cancer treatment. Resistance exists against every effective anticancer drug and can develop by numerous mechanisms including decreased drug uptake, increased drug efflux, activation of detoxifying systems, activation of DNA repair mechanisms, evasion of drug-induced apoptosis, etc. There are contrasting data on a possible correlation between the level of expression of multidrug resistance (MDR) associated drug transporter P-glycoprotein (P-gp) and the susceptibility to complement-dependent cytotoxicity (CDC). Our previous studies revealed that enhanced resistance of chemo-selected MDR ovarian carcinoma cells to CDC is not conferred by P-gp, but due largely at least to overexpression of ACKR2. We here investigated the sensitivity of the human breast cancer cell lines SKBR3 and BT474 to complement-dependent cytotoxicity in response to short-term treatment with Paclitaxel and Doxorubicin. Drug treated carcinoma cells showed increased resistance to CDC associated with overexpression of membrane-bound complement regulatory proteins, CD46, CD55 and CD59. Drug treatment of carcinoma cells also induced the release of the soluble C1 inhibitor and factor I. We conclude that the release of multiple soluble complement regulators into the microenvironment of breast carcinoma cells together with enhanced levels of mCRPs induced by chemotherapeutic agents even upon short-term treatment is significantly increased to mimic tumor cell resistance to complement-mediated killing. This work is financially supported by German Academic exchange service (DAAD), through German Egyptian Research Long-Term Scholarship Program (GERLS).
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
POSTER PRESENTATIONS

P.B2.05.16
Characterization of the chemokine receptor CXCR4 on CDS4+ and CDS5- leukemic cells correlates with favorable prognosis in chronic lymphocytic leukemia
V. Smotkova Kraicova1, G. Manukyan1*, R. Filirova1, Z. Mikulakova1, G. Gabrova1, R. Urbanova2, P. Tursanyi1, A. Petrackova1, T. Papajik1, E. Kriegova1
1Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic; 2Laboratory of Molecular and Cellular Immunology, Institute of Molecular Biology NAS RA, Yerevan, Armenia; 3Department of Hemato- Oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic.

Despite the shared pattern of surface antigen, population of neoplastic cells of neoplastic clones in peripheral blood of CL patients as well as their relevance to the disease progression and patient characteristics. CDS5+ cell subpopulation expressed higher percentage of CCRX (P<0.001), CCRX1 (P=0.007), CCRX10 (P=0.001), and CD62L (P=0.029) compared with those expressed on CDS5- cells, whereas CDS5- cells expressed higher levels of CCK/CK receptor (P=0.001). Accordingly, CCK/CK/CXCR4 ratio was higher on CDS5+ comparing to CDS5- B-cells (P=0.001). Mutated IGSH status was strongly associated with higher percentage of CDS5+ cells on both CDS5+ (P=0.001) and CDS5- (P=0.001), as well as higher MFI of CKR on both CDS5+ (P=0.006) and CDS5- (P=0.005) cells. Combination of CCK/CK (MFI) - CCRX/CXCR4 ratio or CCKR (MFI) - CCRX4 (MFI) was the most significant to discriminate CDS5+ and CDS5- subpopulations. Our results suggest CXCR4 as a marker which greater expression portend a favourable prognosis. Further investigation of CL cell heterogeneity will advance our understanding of neoplastic cell biology and its links to the prognosis.

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Smotkova Kraicova V.*, Manukyan G.*
*contributed equally

P.B2.05.17
Checkpoint inhibitors, cancer and myositis: A report of four illustrative cases
G. Vila-Pijany1, M. T. Sanz-Martinez1, L. Villos-Gimenez1, J. Ros1, J. Lestades-Bardot1, A. Navarro1, F. Martinez-Vale1, V. Garcia-Patos1, C. Carpio1, J. C. Milisenda1, J. M. Grau1, R. Pujol-Borrell1, A. Sela-O'Callaghan1
1Immunology Division, VALL d’Hebron University Hospital (HUHN), Barcelona, Spain, 2Vall d’Hebron Institute of Oncology (VHIO), Vall d’Hebron University Hospital, Barcelona, Spain, 3Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic, 4Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic.

Introduction: Immuno-therapy aimed at blocking PD1/PDL1 pathway restores anti-tumor immunity but may result in a number of Immune-Related Adverse Events (IRAE).

Patients and Methods: Three patients, receiving PD1/PDL1 blockers (Cases 1 to 3) were referred to internal medicine because of symptoms suggestive of myopathy. Case 4 was already diagnosed as dermatomyositis. Clinical, immunological and histopathological evaluation confirmed immune-mediated myositis.

Results: Case 1 was a breast carcinoma who 24 hours after the first cycle of anti-PDL1 (Pembrolizumab) presented with asthenia and a macular rash in both ankles followed 3 months later by neutropenia, hypothyroidism and amyopathic dermatomyositis. Case 2 was a malignant thymoma treated with anti-PDL1 (Atezolizumab) who developed immune-mediated necrotizing myopathy negative for myositis auto-antibodies (16 antigens). Case 3 was a non-Hodgkin lymphoma receiving anti-PDL1 (Nivolumab) who developed sporadic inclusion body myositis with anti-CNI.A. In cases 1 and 2 the suspension of immune checkpoint therapy after 11 and 4 cycles respectively plus steroids induced remission of myositis. In case 3 treatment interruption did not result in improvement. Case 4 was already diagnosed of paraneoplastic dermatomyositis associated to a primary small-cell lung carcinoma positive for anti-NKXP2 prior to anti-PDL1 (Nivolumab). Interestingly anti-PDL1 was well tolerated with a partial response of the tumour without exacerbation of the dermatomyositis.

Conclusion: Immune mediated myositis has been reported in very few patients on checkpoint inhibitor therapy. Skin lesions and muscle weakness should alert and prompt for effective management. On the other hand, the presence of paraneoplastic dermatomyositis is not a contraindication of checkpoint inhibitors treatment.

P.B2.05.18
Identification of a stromal stem cell in pancreatic cancer that drives tumor growth and metastasis
Z. Wu1,2, X. Zhang1, Y. Zhao1, J. Zhou1, A. Selva-O’Callaghan1,2, A. Hennino1,2,3, X. Zhang4,5,6,7
1Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, 2Department of Pulmonology, Semmelweis University, Budapest, Hungary, 3Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary, 41st Dept. of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary, 5Research Institute of Hematology and Cell Therapy, Semmelweis University, Budapest, Hungary, 6Institute of Immunology, Rostock, Germany, 7Institute of Molecular Biology NAS RA, Yerevan, Armenia.

Introduction: Cancer stem cells (CSCs) have been characterized as the properties of stem-like features, tumorigenicity and treatment-resistant. Investigations of CSCs have been manipulated in pancreatic ductal adenocarcinoma (PDAC), in which tumor microenvironment plays an important role in tumor regulation. However, the identification of pancreatic CSCs within native pancreatic tumor microenvironment still remains unclear.Materials and Methods:KC (p48-Cre;K-RasV12) and K14F1P/F1P-Cre; K-RasV12/+; Inka2/ArfWts mice model, cell culture, immunohistochemistry and immunofluorescence, FACS, RT-qPCR, subcutaneous injection into Rag KO mice. Results: A subpopulation of cells in pancreas of K14F1P/F1P-Cre; K-RasV12/+; Inka2/ArfWts mice expressed higher levels of CXCR4 receptor (P<0.001), CXCR5 (P<0.001), CCR10 (P=0.029) compared with those expressed on CD5 low cells. Moreover, this subpopulation shares some features of embryonic stem cells (Oct4/14 and adult stem cells (Nestin). Moreover, Alk4 (Activin A cognate receptor) and Alk5 (TGF-B1 receptor) expression is significantly higher in CD24+CD44+subpopulation compared to CD24-CD44-counterpart suggested the involvement of TGF/Activin signaling. Subsequent analysis of the subpopulation along with Chemokine and Adhesion molecules showed that CXCR4 expression was the key molecule in the progression of the tumour. Conclusion: We also discovered that the cell and spatial density is highly related with their degree of differentiation and outcome both in vitro and vivo.Conclusion: We have identified a population of stromal stem cells generated from KC mice which might be at least in part at the origin of the desmoplastic reaction in PDAC. Further in vivo investigation will provide new insights in the mechanism of generation of the stromal reaction.

P.B2.05.19
Role of carbohydrate metabolism aberration in non-small cell lung cancer-related immunoparalysis
Z. I. Komlasi1,2, G. Szűcs1, E. Imre2, M. Szentkereszty3, G. Barna2, G. Lasonczy2, A. Szántó3
1Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, 2Department of Pulmonology, Semmelweis University, Budapest, Hungary, 3Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary.

Human leukocyte antigen-D (HLA-D) is a major histocompatibility complex class II cell surface receptor, is involved in antigen presentation to T helper cells, and its reduced expression on monocytes is associated with poor prognosis in non-small cell lung cancer (NSCLC). Also elevated fasting serum glucose level (>7 mmol/L) and diabetes mellitus is independent prognostic markers in NSCLC (Lung Cancer 2012; 76:242), however the mechanisms by which the abnormality of carbohydrate metabolism aggravate lung cancer have not been fully clarified. Hyperglycemia and insulin resistance are known to induce a reduction in HLA-D expression (imunoparalysis) in sepsis. We aimed to investigate the role of carbohydrate metabolism abnormality in NSCLC-related immunoparalysis. 33 NSCLC patients (stage IIIb-Iv) were included in the study. Monocyte HLA-D expression was measured by using flow cytometry. Serum glucose and insulin concentration was measured and Homeostasis Model Assessment - Insulin Resistance 2 (HOMA-IR2) score was calculated. 18 patients had hyperglycemia, and 16 out of them were insulin resistant (HOMA-IR2 ≥ 2). A significant indirect correlation was observed between both insulin expression, and between HOMA-IR2 and HLA-D IR expression. Our results suggest that insulin resistance may contribute to the unfavorable alteration of antigen presentation capacity, and consequently, an insufficient anti-tumor immune response in NSCLC. This underline the previously unappreciated importance of the management of carbohydrate homeostasis in lung cancer patients. Funded by NKFIH K 108009.

P.B2.05.20
Monitoring antibody responses against tumors associated protein modified peptides might clinically be of prognostic relevance
H. Thiesen1, E. Steinbrecher1, E. Schade2, M. Maruschke1,2, E. Kriegova1
1Institute of Immunology, Rostock, Germany, 2Gesellschaft für Individualisierte Medizin, Rostock, Germany, 3Heilas Hanselinklinik Stralsund, Stralsund, Germany, 4Department of Urology, Mecklenburg-Rostock, Rostock, Germany.

Introduction: Whole somatic genome sequences of three patients suffering from clear cell renal cell carcinoma (ccRC) have been computationally analysed to categorize protein structures mutated in these tumors. The working hypothesis addressed is focused on the prognostic relevance (harmful or beneficial) of elicited tumor-associated antibodies reactivity. Method: Peptides representing putative neoepitopes such as point as well as frame shift mutations were synthesized. Peptide microarrays were used to study mutated peptides that show epitope-antibody-reactivities (EAR) in sera of ccRC patients. The ten most informative peptides were finally subjected to MSD multi-arrays by addressing 10 different peptides per well. Results: Of 94 ccRC sera tested, 20 sera showed at least one positive signal, of which 8 sera reacted with more than one peptide selected from our initial screen. Interestingly, the patient that show EAR with all 10 preselected peptides turned out to suffer from metastatic ccRC. He died shortly after his tumour had been removed. Conclusion: Our multi-array analysis of tumour associated antibodies directed against mutated peptide sequences present in patients suffering from clear cell renal carcinoma indicate that the presence of antibodies directed against tumour related protein structures might have a negative prognostic effect.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
265
Tumor-associated neutrophils (TANs) influence tumor growth and angiogenesis, depending on IFNs availability in milieu. Since angiogenesis plays a crucial role in tumor progression, the aim of this study is to determine the mechanism responsible for neutrophil-dependent tumor vasculization, and how IFNs impact this process. Methods: First, we analyzed TAN infiltration, vasculature development and maturation stages of endothelial cells in the tumors. We assessed also hypoxic areas and the vessel leakiness in tumors. Pro-angiogenic regulatory gene expression was evaluated in endothelial cells after stimulation with TANs. Additionally, we assessed the capacity of TANs to stimulate proliferation, tube formation assay and sprouting formation capacity of endothelial cells. To delineate the role IFNs play in the regulation of tumor angiogenesis, we compared TANs from IFN-α deficient mice with WT. Results: We could observe elevated tumor growth and higher TAN numbers in IFN-α deficient mice which were associated with more mature, functional phenotype tumor vasculature and show significant upregulation of pro-angiogenic factors, such as Neuropilin1 (NRPI) and Endoglin (ENG). Interestingly, these tumors show also increased hypoxia. Co-culture of endothelial cells with such proangiogenic IFN-α deficient TANs led to profound increase of their proliferation, migration, tube formation and sprouting capacity. In agreement, aortic ring assay showed more microvessel outgrowths after addition of IFN-α deficient TANs. Conclusion: Taken together, our results suggest that IFN-α deficient TANs of pro-angiogenic phenotype can efficiently stimulate tumor vascularization and tumor growth. The lack of type I IFNs stimulates their proangiogenic capacity due to upregulation of Pro-angiogenic regulatory genes.

P.B2.06.02
Peroxidase activity on MCF-7 cells may be improved by propolis without affecting the viability of human monocytes

E. O. Cardoso
1Graduate Institute of Oncology, College of Medicine, National Taiwan University, Taipei, Taiwan, 2Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan

Introduction: Cancer is a disease that affects millions of people in different continents, resulting in millions of deaths annually. Tumor angiogenesis has been used to treat breast cancer and considering the side effects caused by anticancer agents, such as toxicity, induction of resistance in tumor cells and immunosupression, the administration of natural products simultaneously with anticancer drugs has been investigated. Propolis is a bee product displaying a cytotoxic activity against tumor cells and modulatory effects on immune cells. This study assessed the cytotoxic effects of propolis in combination with tamoxifen (P + TAM) on MCF-7 cells and on human monocytes. MCF-7 cells were used as a tumoral model and monocytes were treated with PMA. The expression of genes of interest was quantitated by Syber Green Real-Time PCR method. Materials and Methods: RAW 264.7 and PMA-treated THP-1 cells were primed with IL-4, IL-10, IL-13 and TGF-b, and were treated with tamoxifen (0.25, 0.5, 1 and 2.5 μM), propolis (25, 50, 75 and 100 μg/mL), and their combinations for 24, 48 and 72 h. Human monocytes were obtained from healthy donors and treated with the same concentrations for 18 h. After these periods of time, cell viability was assessed by the colorimetric MTT assay. Significant differences between treatments were determined by analysis of variance (ANOVA), followed by Dunnett’s test (P < 0.05). Among all treatments and concentrations, propolis (50 and 75 μg/mL) increased the cytotoxic action of tamoxifen (0.25 and 0.5 μg/mL) against MCF-7 cells after 48 h without affecting monocyte viability. This finding indicated that these combinations may be efficient against tumor cells in vitro but not normal ones, preserving monocytes functions. Financial support: HAPESP 2016/09886-4.

P.B2.06.03
IFN-γ-signaling involved in PD-L1 expression in mouse associated M2 macrophages of orthotopic liver cancers treated with sorafenib

C. Chang1, S. Yang1, C. Hsu2
1Graduate Institute of Oncology, College of Medicine, National Taiwan University, Taipei, Taiwan, 2Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan

Introduction: We previously demonstrated that PD-L1 expression increased in tumor-associated macrophages (TAMs) of hepatocellular carcinoma (HCC) tumors progressing from sorafenib treatment. The current study explored the potential mechanisms underlying the increased PD-L1 expression in TAMs of HCC. Materials and Methods: BNL cells, a mouse liver cancer cell line, were implanted on liver of BALB/c mice, and were fed with sorafenib (5 mg/kg/day) for 1 week. Specific antibodies were used to detect the expression of PD-L1, F4/80, CD68, or MHC II in TAMs. RAW 264.7 and PMA-treated THP-1 cells were primed with IL-4, IL-10, IL-13 and TGFB, followed by treating IFN-γ plus TNF-α or LPS. The expression of genes of interest was quantitated by real-time PCR method. Results: Sorafenib treatment suppressed the growth of BNL mouse liver tumors. CD11b+F4/80+ TAMs (50-73%) from sorafenib-treated liver tumors exhibited increased expression of PD-L1 (MFI 316.8), CD86 (MFI 136.3), and MHC II (MFI 341.7). Transcripts analysis not only confirmed the increased expression of PD-L1, but also revealed the upregulation of IFN-γ-signaling including IFOS, IL-12, Stat1, IRF1, and JAK/12 in TAMs from sorafenib-treated liver tumors. In cultured RAW 264.7 or THP-1 cells with M2 polarization, we found that IFN-γ+ TNFα or IFN-γ+ LPS upregulated the expression of PD-L1, CD86, and MHC II. Conclusion: Our data suggest that activation of IFN-γ-signaling contributes to PD-L1 upregulation in TAMs of mouse liver cancer treated with sorafenib.

P.B2.06.04
Food-derived β-glucans: polarization towards M1-like macrophages

P. de Graaff1; +, A. van Laa1r, M. M. Tomassen1, C. Berrevoets2, R. Debets2, C. Govers1
1Erasmus MC-Cancer Institute, Rotterdam, Netherlands, 2Food & Biobased Research, Wageningen, Netherlands

Introduction: Immune therapies have shown clear clinical effects in the treatment of solid tumors. Despite significant initial responses, these therapies are currently challenged by incomplete and non-durable responses in the majority of patients, which are in part related to T cell evasive mechanisms. In the current study, we assess non-digestible polysaccharides (β-glucans) for their ‘adjuvant effect’ towards innate immune cells to support anti-tumor effects of T cells. Materials and methods: Nine β-glucans (Maitake D-fraction, Oat, Zymosan, Lentinan, Curdian, Schizophyllan, Whole Glucan Particles and two types of yeast-derived β-glucans) were tested for their effects on macrophages to determine which macrophages (M0) or macrophages that were first differentiated into macrophages (M1) or macrophages that were first differentiated into macrophages (M2) were more susceptible to polarization. The phenotype and function of resulting macrophages was assessed by flow cytometry analysis, real-time PCR and Western blot. Results: Zymosan, Yeast Inmune and Curdian up-regulated gene expression of CCR7, ICAM-1 and CD80, and significantly increased the secretion of both TNFα and IL-6 in M0 macrophages. Notably, when starting from immune suppressive M2-like macrophages, often prevalent in solid tumors, these three β-glucans again pushed macrophages towards an M1-like polarization. Moreover, these β-glucans induce expression of T cell selective chemokine receptors, and were able to reverse macrophage-mediated inhibition of anti-tumor T cell responses. Conclusion: These in vitro analyses demonstrate that selected β-glucans have the unique ability to preferentially skew macrophages toward an M1-like, T cell supportive phenotype.
Characterization of novel CD73 antibodies as a therapeutic method of adenosine regulation

G. Gerson,1 S. Grooby,1 L. Tonkin,1 A. Bitterwolf,1 L. Stewart,1 P. Shah,1 Z. Johnson,1 K. Ewing2
1Cancer Research UK Therapeutic Discovery Laboratories, Cambridge, United Kingdom, 2Cancer Research UK Therapeutic Discovery Laboratories, London, United Kingdom.

CD73 is a membrane-bound nucleotidase receptor which is frequently overexpressed in the tumour microenvironment and can be found on both tumour and infiltrating immune cells. Its function is to catalyse the conversion of adenosine monophosphate (AMP) to adenosine and phosphate and it has been proposed as a therapeutic target in cancer due to the role of adenosine in tumour immune suppression.

A series of CD73 antibodies were characterised in vitro using multiple approaches. Inhibition of CD73 activity was evaluated using an Amplex Red-based coupled adenosine assay against both human and mouse CD73, and kinetics of antibody binding were determined using BioLayer Interferometry. Cellular assays were then utilised to further evaluate the antibodies in vitro. The ability of the CD73 antibodies to internalise was evaluated using two different methods, a Fab-ZAP killing assay and the IncuCyte™ FidFluor internalisation assay. The antibodies have also undergone functional studies that investigate the ability of the CD73 antibodies to disrupt the production of adenosine in tumour cells.

We demonstrate that amongst our panel of antibodies inhibit CD73 function by two different mechanisms, direct inhibition of enzyme activity and modulation of cell surface expression; both of which have therapeutic potential to disrupt CD73-mediated adenosine production and therefore reduce anti-tumor immune responses. Several antibodies from this panel will be advanced into late-stage preclinical development to identify a clinical development candidate.

Expression of the P2X7R(K) splice variant by 4T1 breast cancer cells inhibits tumor growth in vivo

S. Javed, F. Koch-Nolte, F. Haag;
University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Immunogenic death caused by chemotherapy can favor T cell activation by the release of damage-associated molecular patterns, including ATP. Various tumor and immune cells express the ATP-gated P2X7 ion channel. Activation of P2X7 on tumor cells benefits tumor growth by stimulating cell metabolism, but strong stimulation of P2X7 can kill tumor cells. P2X7 expression by host tumor cells is detrimental to the tumor because it enhances the anti-tumor immune response. Little is known about the role of P2X7 in the context of chemotherapy. We therefore studied the effects of P2X7 stimulation on Doxorubicin (DOX) toxicity in lymphoma and breast cancer cell lines. Low doses of extracellular ATP (eATP) synergistically enhanced the sensitivity to DOX. This effect was linked to a specific splice variant (P2X7(k)) of P2X7, and was further enhanced by an agonistic nanobody to P2X7. Mechanistically, gating of P2X7 augmented the initial uptake of DOX into cells. However, enhanced cell death was also observed when DOX was washed away before exposure to eATP, suggesting that the synergism resulted from an interaction of downstream signaling pathways. In the 4T1 breast cancer model expression of P2X7(k) inhibited tumor growth in vivo by increasing apoptosis and enhancing immune cell infiltration. Our results suggest that expression of P2X7(k) by tumor cells may contribute to their sensitivity to chemotherapeutic drugs.

Hypoxya regulates the fate of y T cells in tumour microenvironment

S. Kashapathi Sureshbabu1,2, A. D’Cruz1, D. Chaukar1, S. V. Chiplunkar1,2
1Advanced Centre for Treatment, Research and Education in Cancer, Navi-Mumbai, India, 2Homi Bhabha National Institute, Mumbai, India.

Oral cancer is the most common cancer in India with relatively poor prognosis. Hypoxia is one of the factors important in predicting survival, contributing to tumour progression, therapy resistance and poor clinical outcome. Understanding the complexity of tumour microenvironment (TME) is important for the development of immunotherapy. yT cells infiltrate tumours and exhibit potent antitumor activity, hence are becoming the attractive candidates for cancer immunotherapy. In the current prospective study, we aimed at investigating the effect of hypoxia on the effector functions of y T cells. yT cells showed increased oral tumor infiltration, exhibited marked differences in the expression of activation markers CD69 and CD25, effector molecules perforin and IFNγ. Enhanced expression of hypoxia inducible factor-1α was observed in yT cells in oral tumours. Freshly isolated yT cells from healthy individuals were stimulated with ccDMaB and 1-Hydroxy-2-methyl-2-butene-4-y1-diphosphate (HDMAPP) and cultured in the presence or absence of PGE2. Under hypoxia, antigen specific proliferation and activation status of yT cells was unaltered. A marked decrease was observed in the anti-tumour cytotoxic activity of yT cells owing to the decreased expression of IFNγ, CD107a and transcription factors Eomes and Tbet which are responsible for regulating the cytotoxic effector functions. Under hypoxia, yT cells express increased ROS and secreted cytokines favouring yT17 differentiation. Gene expression studies of hypoxia exposed yT cells confirmed yT17 differentiation to a pro-tumor phenotype promoting angiogenesis. In conclusion, we demonstrate that hypoxia TME gives survival advantage to yT17, thus promoting immune evasion.

Targeting Myeloid-Derived Suppressor Cells (MDSC) in Myelodysplastic Syndromes (MDS).

J. Liu, X. Chen, E. Eksioglu, S. Wei;
Moffitt Cancer Center, Tampa, United States.

The acquisition of genetic alterations that lead to ineffective hematopoiesis is a characteristic of MDS. This event is associated with inflammatory bone marrow (BM) microenvironment; however, the underlying mechanisms are unclear. We have identified that myeloid derived suppressor cells (MDSC) are key driver of MDS progression. We found that MDSCs accumulated in excess in the BM of patients with MDS compared to controls and non-MDS cancer patients. We then performed to determine whether MDSCs represent a distinct cell population from the abnormal MDS clone. MDSs chromosomal abnormality were separated by FACS sorting based on MDS phenotype and the presence of chromosomal abnormalities was determined in this population and compared to non-MDSs. Chromosomal abnormalities resided within the non-MDS hematopoietic compartment indicating that MDSCs in MDS patients may represent a unique cell population from the HPC with clonal potential. Furthermore, the key cytokines involved in MDS suppressive function were higher in MDS patients compared to controls. It was observed that the accumulation of MDSC in the BM from MDS patients has an impact on hematopoietic differentiation. Based on these findings, a novel form of adaptive immunotherapy based on the induction of MDS maturation can be envisioned. DAP12, an adaptor protein, mediates signaling of myeloid cell maturation, were genetically modified to be a constitutively activated form. Infection of BM-MNC from MDS patients with constitutively active DAP12 increased expression of maturation surface markers and increased BFI-E colony formation after 14 days. These results suggest that activation of DAP12 has potential implications in MDS.

Epigenetic alteration of the PD-1/PD-L1 axis, a novel target for pharmacotherapy?

D. McKernan, C. Hennessey, F. Quirke, G. O'Malley, A. Ryan;
National University Ireland Galway, Galway, Ireland.

Introduction: Programmed death ligand 1 (PD-L1) is the primary ligand of the receptor programmed death-1 (PD-1), a co-inhibitory cell surface immunoglobulin important in development of self-tolerance. PD-L1 expression can be exploited by various cancers as a means of immune evasion with high PD-L1 expression associated with a poorer prognosis. Checkpoint inhibitors which target the PD-1/PD-L1 axis have shown some promise in the clinic but there is a lack of knowledge on the precise mechanisms by which PD-L1 inhibition is beneficial. Genes expression is regulated by known to alter gene expression, we therefore hypothesized that the DNA methylation and/or histone acetylation may alter PD-L1 expression. Methods: We used the colorectal cancer HCT116 cell line with genetically deleted DNA methyltransferase (DNMT) enzymes to determine the effect of DNA methylation on basal mRNA by qPCR and protein expression by flow cytometry. Using a series of microsatellite instable human colonic carcinoma cell lines to induce PD-L1 expression with the TLR3 ligand Poly I:C (10 μg/ml, 24 hrs) we determined the effect of both DNMT (decitabine 500 nM, 72 hrs) and histone deacetylase (SAHA, 10 μM) inhibitors on expression with the TLR3 ligand Poly I:C (10 μg/ml, 24 hrs) we determined the effect of both DNMT (decitabine 500 nM, 72 hrs) and histone deacetylase (SAHA, 10 μM) inhibitors on expression. Results: Genetic knockout of DNMT enzymes significantly reduced the basal expression of PD-L1. Decitabine but not SAHA prevented Poly I:C induced upregulation of PD-L1 mRNA and protein. Using a NF-κB superactivated (3R) cell line, we determined that PD-L1 induction was NF-κB dependent and decitabine treatment prevented this induction. Conclusion: These data suggest that epigenetic modifying drugs may act as immunosuppressors and potentially form part of future PD-1/PD-L1 therapies.

Development of a syngeneic mouse model of leukemia minimal residual disease: a new tool to study the involvement of the immune response in cancer cell persistence and test new immunotherapeutic strategies

A. MOOW, B. QUINSEL, C. BRINSTEIN

Acute myeloid leukemia (AML) is a clonal disorder characterized by blocked differentiation and extensive proliferation of hematopoietic progenitors/precurors. Relapse is often observed after chemotherapy due to the presence of residual leukemia cells, also called minimal residual disease (MRD). Several studies have demonstrated that sub-clonal heterogeneity at diagnosis can be responsible for MRD, with major or minor sub-clones resistant chemotherapy or emerging after treatment. However, these studies do not provide information about the contribution of the immune system (elimination, control or escape) in these leukemic cell persistence or sub-clones hierarchy.
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P.B2.06.11
The gastrointestinal tract tumour microenvironment differentially influences maturation of and cytokine secretion from dendritic cells
M. Morrissey1, M. Dunne1, R. Byrne1, N. Lynam-Lennon1, S. Kennedy1, C. Nulty1, N. McCabe1, C. Butler2, D. O'Toole3, E. Pear1, J. V. Reynolds4, J. O'Sullivan1
1Trinity College Dublin, Dublin, Ireland, 2University College Dublin, Dublin, Ireland, 3St James's Hospital, Dublin, Ireland.

Oesophageal adenocarcinoma (OAC) and rectal adenocarcinoma are treated with neoadjuvant chemoradiotherapy in order to reduce tumour size prior to surgery however only 10-30% of patients have a complete pathologic response. Inflammatory and angiogenic mediators in the tumour microenvironment (TME) have many functions, such as enabling evasion of anti-tumour immune responses by disabling infiltrating dendritic cells (DCs) and have been linked with radiosensitivity. Tumour Conditioned Media (TCM) from colonic cancer has been shown to strongly inhibit DC maturation. Our aim was to understand if this DC inhibition extends to other cancers of the gastrointestinal tract, to investigate if radiotherapy influences this and to profile constituents of TCM that may influence DC maturation.

Here we found that monocyte-derived DCs remained responsive to LPS following pre-treatment with OAC cell line TCM, whereas inhibition was induced by CRC cell line TCM. Ex vivo TCM from different gastrointestinal adenocarcinoma types induced different effects on DC maturation with oesophageal inducing DC activation, rectal inducing minor activation and colonic inducing inhibition of DC maturation. Interestingly, all cancer types induced DC inhibition of secreted TNF alpha. It was also found that 26gy irradiated TME induced significant inhibition of DC maturation for irradiated rectal adenocarcinoma and no effect with irradiated oesophageal cancer. Differential levels of inflammatory (IL2) and angiogenic mediators (Ang2 and bFGF) in TCM of GI tumours correlated with DC maturation.

Overall we found that there are differences in the human TME from different gastrointestinal cancers which can directly impact various levels of inhibition of LPS-induced DC maturation.

P.B2.06.12
Investigation of the roles of anti-VEGFR1 natural antibodies in human plasma in hepatocellular carcinoma
C. Rodgers, A. Pritchard, J. Wei; University of the Highlands & Islands, Inverness, United Kingdom.

Natural antibodies have been found to serve as an important tumorigenic system in the body and their anti-tumor cytotoxicity has been confirmed in vitro study. Natural antibodies are defined as the immunoglobulins produced by B1 lymphocytes in the absence of exogenous antigen stimulation. They are physiologically involved in maintaining tissue homeostasis such as clearance of apoptotic cell debris, as well as elimination of invading pathogens as well as destruction of cancer cells formed in the body. In recent studies, it was found that natural IgG antibodies against vascular endothelial growth factor receptor 1 (VEGFR1) present in human plasma could be used to treat patients with hepatocellular carcinoma (HCC).

This study was thus designed to look at whether different epitopes derived from the VEGFR1 extracellular domain render HCC cells to have different responses to natural anti-VEGFR1 IgG in human plasma. An enzyme-linked immunoassortment assay was developed in-house with synthetic VEGFR1-derived peptides to screen human plasma rich in anti-VEGFR1 IgG that was applied to test the inhibitory effects of natural anti-VEGFR1 IgG on proliferation of HCC cell lines. Anticancer mechanism by which natural anti-VEGFR1 IgG kills HCC cells will be also investigated through analysis of cell viability, apoptosis, autophagy, gene expression, luciferase reporter assay, wound-healing and transwell migration.

P.B2.06.13
Alterations of the immune environment of Sezary cells
M. Roelens1, C. Ram Wolff2, M. Delorda1, G. Maki1, A. Marie-Cardine1, A. Bensussan1, M. Bagot1, A. Toubert1, H. Moins-Teissèrec1
1INSERM UMR1630, IUMH, AP-HP, Paris, France; 2INSERM UMR976, UFR de Medecine, Paris, France.

Introduction Sézary syndrome (SS) is a leukemia aggressive form of Cutaneous T-cell lymphomas. A challenge in SS is the preservation of antitumor and anti-infectious immunity, as infections are the most frequent cause of lethality. We previously defined the CD158k/KIR3DL2 molecule as a positive “generic” cell-surface marker for Sézary cells (SCs), and found an unexpected heterogeneity of naive/memory subsets. Such marker allows the characterization of non-SCs. Our aim was to analysis the phenotypes and functions of the “benign CD4+ T-cell counterpart” as well as other components of the innate and the adaptive immunity in the context of SS. Methods: Blood samples from patients and skin biopsies collected the same day, were analyzed using flow-cytometry for T cell differentiation markers, resident-memory markers, as well as skin-homing, interleukins receptors and immune-checkpoint molecules. TCR repertoire was analyzed for 3 patients. Patients’ derived monocyte subsets were analyzed both phenotypically and after maturation and immune-checkpoint molecules. TCR repertoire was analyzed for 3 patients. Patients’ derived monocyte subsets were analyzed both phenotypically and after maturation and differentiation into dendritic cells in proliferation assays. Results: We show that the “benign CD4+ T-cell counterpart” of SCs shares phenotypic similarity with malignant cells. These cells do not express antigens and cytokines and repertoire with enhanced expression of exhaustion molecules and defects in proliferative capacities. These abnormalities are not confined to the CD4+ T-cell subset, as monocytes show a significant downregulation of HLA-DR molecules. Conclusion: Our results are in favor of profound alterations of the immune environment of SCs at phenotypic and functional levels. We are currently examining the relationships between SCs and the non-malignant immune compartment in both blood and skin compartments.

P.B2.06.14
Hypoxia effect on macrophages activation and fibroblasts cross-talk in chronic inflammatory and tumor microenvironment
E. Setter1, M. Locatelli2
1Humanitas Clinical and Research Center, Rozzano (MI), Italy, 2Department of Medical Biotechnologies and Translational Medicine, Università degli Studi di Milano, Segrate (MI), Italy.

Macrophages (MΦ) are highly plastic cells, able to assume different functional phenotypes depending on the microenvironment. To investigate the relevance of different microenvironmental cues on MΦ activation, we generated in vitro MΦ with an inflammatory phenotype using LPS+IFNγ (M1) or with an anti-inflammatory and pro-tumoral environment of SCs at phenotypic and functional levels. We are currently examining the relationships between SCs and the non-malignant immune compartment in both blood and skin compartments.

P.B2.06.15
Targeting PD-1/PD-L1 Pathway in tumor Microenvironment of Sebaceous Gland Carcinoma as a New Paradigm of Immunotherapy
L. Singh1, M. K. Singh1, S. Kashyap1, S. Sen1, M. A. Nizvi2
1Jamia Millia Islamia, New Delhi, India, 2All India Institute of Medical Sciences, New Delhi, India.

Introduction: Sebaceous gland carcinoma (SGC) is a malignant eyelid cancer and exhibit aggressive behaviour as it metastasizes to lymph nodes and distant organs. Understanding tumor-stromal interaction in tumor microenvironment has led to the development of immunotherapy in SGC. Programmed cell death-ligand 1 (PD-1/PD-L1) interaction negatively regulates T cell activity and helps in the tumor escape mechanism. The aim of this study was to analyze PD-1/PD-L1 expression in tumor microenvironment as a prognostic marker and as a possible therapeutic target for SGC. Methods: Expression of immune markers (PD-1 and PD-L1 protein) was evaluated in 52 prospective cases of sebaceous gland carcinoma by immunohistochemistry and qRT-PCR. Results were correlated with clinicopathological parameters and patient outcome by statistical analysis. Results: Histopathological analysis revealed that 19 (36.5%) tumours were poorly differentiated and pagetoid spread was present in 22 cases. Immunoreactivity of PD-1 and PD-L1 expression was found in tumor and stroma cells. PD-L1 showed immunoeexpression in 51.92% cases whereas PD-1 showed positive expression in 34.61%. PD-L1+ was more common than PD-L1+ expression.

P.B2.06.16
Panitumumab induces anti-angiogenic activity in vitro and in vivo in colorectal cancer
O. Donnelly1, S. K. Nulty1, J. V. Reynolds1, C. Nulty1, N. McCabe1, D. O'Toole2
1INSERM UMR-976, UFR de Medecine, Paris, France; 2INSERM UMR-976, UFR de Medecine, Paris, France.

Panitumumab (Pan) is a fully human monoclonal antibody that targets EGFR and induces anti-angiogenic activity in vitro and in vivo. We investigated whether Pan is able to induce anti-angiogenic activity both in vitro and in vivo in colorectal cancer cells. We tested different conditions of Ara-c administrations (concentrations, number and timing of injections). One protocol led to approximately 50% survival over an average of 90 days after non-treated control mice succumbed to AML (mean survival, 29-31 days). The assessment of residual leukemic cells in the blood of surviving mice at different time points (between 49 to 75 days after control mice died from AML) revealed the presence of 0.1 to 4.3% circulating ZsGreen+ cells by flow cytometry without any signs of AML relapse. Thus, we have developed a mouse model of leukemia MDR that should offer valuable insights into the biology of residual leukemic cells and the immune mechanisms leading to their persistence, thereby enabling the development of new therapies.
POSTER PRESENTATIONS

On univariate analysis, pagetoid spread, lymph node metastasis and tumor expressing PD-L1 expression was associated with a reduced disease-free survival (P=0.005). However, on multivariate analysis, only the PD-L1 expression was associated with worse prognosis (P = 0.031). Conclusion: This is the first of its kind study, which states the role of tumor microenvironment in mediating the immune response and tumor progression of SGC. This paves the way for development of immunotherapy as a new strategy for treatment of metastatic sebaceous gland carcinoma patients.

P.B2.06.16 Interaction of NKp-1 protein Expression with Immunological Microenvironment of Uveal Melanoma and its Prognostic Significance

K. M. Singh, S. Kashyap, N. Pushker, S. Sen, R. Meel, K. Chadoor, S. Bakhshi, J. Kaur; 1AIIMS, New Delhi, India, 2AllIMS, New Delhi, India.

Introduction: Uveal melanomas as malignant phenotype having a high density of macrophages, blood vessels and T-lymphocytes along with the presence of epithelioid cells and high melanin pigmentation which might be related to worse prognosis. High densities of inflammatory cells in uveal melanoma are associated with poor prognostic factors. NKp-1 plays an important role in inflammation which promote cancer initiation and progression. The aim of the study is to detect NKp-1 expression in the immunological microenvironment of uveal melanoma and its prognostic significance. Method: Evaluation of NKp-1 expression was assessed by using immunohistochemistry and western blotting in 75 formalin fixed uveal melanoma tissues and transcriptional analysis was done on 58 frozen fresh tissue by real time pcr. Results: were then correlated with clinicopathological parameters. Results: Out of 75 cases, 40 cases showed both nuclear and cytoplasmic expression and 15 showed cytoplasmic only. qPCR showed upregulation of NKp-1 gene in 72.41% cases at transcriptional level. Expression of both cytotoxic and nuclear-c REL protein showed significant correlation with cases having high tumour infiltrated lymphocytes, macrophages (CD68+) and presence of blood vessels (CD34+). There was a statistically significant difference in the overall survival of patients with nuclear/cyttoplasmic NKp-1 immunopositivity (p=0.05). Conclusion: This preliminary data suggests that NKp-1 protein might play a role in the immunological microenvironment of uveal melanoma which is responsible for the pathogenesis of this disease. Further translational studies are required to explore the nature of NKp-1 interactions in tumour microenvironment of uveal melanoma.

P.B2.06.17 Regulation of the expression of IL-1R8, a regulatory member of the Interleukin-1 receptor family

D. Supino, C. Perucchini, M. Molgora, A. Ponzetto, S. Di Marco, E. Magrini, S. Carnelijke, F. Gianni, S. Jaillon1,2, A. Mantovani1,2, C. Garlanda1,2

1Humanitas Clinical and Research Center, Rozzano, Italy, 2Humanitas University, Rozzano, Rozzano, Italy.

IL-1R8 is an Interleukin-1 receptor (ILR) family member which activates an anti-inflammatory program by inhibiting ILR and Toll like receptor (TLR) signaling. In NK cells IL-1R8 acts a co-receptor to regulate and control viral infections. The purpose of this study was to dissect the regulation of IL-1R8 expression in leukocytes. qPCR-q analysis suggested that Colony Stimulating Factors (CSF)-inducible Transcription Factors and epigenetic modifications affect IL-1R8 in NK cells and macrophages along maturation and activation. IL-1R8 expression was down-regulated during macropage differentiation. Pro-inflammatory molecules involved in macrophage and NK cell activation down-regulated IL-1R8 expression in human and mice. Prostaglandin E2 and Interleukin-10 (IL-10), which are involved in cancer-associated immunosuppression, counteracted this effect in NK cells and up-regulated IL-1R8 in human monocytes and macrophages. RNA-seq analysis, qPCR and Western Blot also revealed the existence of IL-1R8 truncated forms in human immune cells constituted by the exons coding for the intracellular part of IL-1R8, after M1 polarization. Thus pro-inflammatory stimuli are implicated in conventional isoform IL-1R8 down-regulation, overexpression of truncated forms of the protein, whose biological role is presently unknown. In addition, the up-regulation of IL-1R8 in NK cells by PGE2/LT-10 axis suggests that IL-1R8 could be part of the immunosuppressive activity of these molecules. IL-1R8 acts as a checkpoint molecule tuning antitumor and antiviral NK cell activity. Understanding how IL-1R8 expression in NK cells is affected by the tumor microenvironment is essential in the development of this checkpoint as a potential immunotherapy target.

P.B2.06.18 Patterns of immune checkpoint expression by primary tumor cells and tumor infiltrating lymphocytes across different tumor entities

M. Thelen, A. Lechner1,2, K. Wennholz, D. Pfister, F. Döbeli, M. Heldwein, K. Heimatk, D. Reuten1, M. Mallmann, F. Thangarajah, C. Brun1, M. von Bargwelt-Baldini1,2, H. A. Schläfgen1,2

1Cologne Center for Molecular Medicine, Cologne, Germany, 2Department of Head and Neck Surgery, LMU, Munich, Germany, 3Department of Urology, University of Cologne, Cologne, Germany, 4Department of Cardiac and Thoracic Surgery, University of Cologne, Cologne, Germany, 5Department of Head and Neck Surgery, University of Göttingen, Göttingen, Germany, 6Department of Gynecology Surgery, University of Cologne, Cologne, Germany, 7Department of General, Visceral and Cancer Surgery, University of Cologne, Cologne, Germany, 8German Cancer Consortium (DKTK), Heidelberg, Germany, 9Department of Internal Medicine III, LMU, Munich, Germany.

Immune-checkpoint inhibition (CIK) demonstrated breakthrough therapeutic efficacy in several kinds of cancer. These therapies are unique, as the primary target is not the tumor itself, but the crosstalk between immune cells and cancer cells in the tumor microenvironment. Efficacy of CIK is not limited to patients with expression of the respective protein on tumor cells and recent publications demonstrated that expression of PD-L1 on tumor-infiltrating lymphocytes (TIL) can be of similar importance. Expression patterns of 30 described immune checkpoint and regulatory molecules were analyzed on T, B and NK cells in peripheral blood and single cell suspensions of primary tumor samples of nine different tumor entities using flow cytometry. Expression of the respective ligands on primary tumor cells was assessed in tissue microarrays. The majority of analyzed immune checkpoint pathways could be detected. Despite the variety of primary tumor sites, our analyses revealed similar expression patterns for most proteins included in this study. For example, PD-L2 as well as PD-L1 were detectable on tumor cells and tumor-infiltrating lymphocytes in all analyzed tumor entities and expression patterns on TILs were largely overlapping. In addition, we correlated immune checkpoint expression to the infiltration by lymphocytic subsets including regulatory T cells. Immune escape is a common feature of cancer and the specific expression patterns described in this study are of translational relevance for ongoing and future immunotherapeutic trials.

P.B2.06.19 Potential y8 T cell transdifferentiation into a T cells in transplanted children

A. Zorzoli, G. Barbaro, P. Merli, F. Antonini, A. Beritani, E. Ferretti, F. Frassoni, F. Locatelli, I. Airold1,2

1Department of Newborns and Pediatric Cardiac and Thoracic Surgery, University of Genova, Italy, 2Department of Pediatric Surgery, University of Genova, Italy.

We recently demonstrated that pediatric patients with acute leukemia receiving a graft depleted of y8 T and CD19+ B lymphocytes and treated with zoledronic acid (ZOL) showed: i) increased cytotoxicity of y8 T cells against leukemia blasts, ii) rapid reconstitution of y8 T cells and iii) a decrease of GVHD incidence. These data suggested that y8 T cells, reconstituted or infused with the graft, could induce y8 T cell reconstitution. In this study, it has been reported that the specific population of V61+CD4+ y8 T cells can trans-differentiate into y8 T cells, through specific steps of TCR rearrangement. Thus, we hypothesized that ZOL could be an appropriate stimulus for the process of trans-differentiation of y8 T into y8 T cells in transplanted patients. In order to validate this hypothesis we started a set of experiments in order to characterize T cells from transplanted patients infused and not with ZOL. Furthermore, we tested whether human y8 T cells can transdifferentiate in a T cells using high immunodeficient mice NOB/SCID/IL2rg-/- (NSG). Preliminary results showed that in transplanted patients treated with ZOL the population of V61+CD4+ y8 T cells appears, and that NSG mice may represent a suitable model to recapitulate trans-differentiation of y8 T into y8 T cells. Further investigations are needed to confirm that the trans-differentiation of y8 T into y8 T cells may be an innovative immunotherapy against pediatric acute leukemia.

GRANT: A.I.R.C. Ig 17047

P.B2.06.20 CD117 following CD8+ T cell priming and stratifies sensitivity to apoptosis according to strength of initial engagement

G. Frumento, J. Zuo, K. Vermani, G. Moss

Institute of Immunology and Immunotherapy, Birmingham, United Kingdom.

CD117 (Kit) is the receptor for stem cell factor (SCF) and plays an important role in the development of early thymocytes, but it was not known to be expressed downstream the triple negative stage. When we found CD117 transcripts in activated mature T cells, we studied the characteristics of the phenomenon. We found that CD117 is expressed following priming of mature CD8+ naïve T cells in vitro and is detectable in vivo in CD8+ T cells following primary Epstein-Barr virus infection. CD117 expression is mediated through an intrinsic pathway and is suppressed by IL-12. Importantly, the extent of CD117 expression is inversely related to the strength of the activating stimulus and subsequent engagement with costimulatory molecules SIgF markedly increases susceptibility to apoptosis. CD117 is therefore likely to shape the population of T cell immunodominance during a prime immune response by rendering cells with low avidity for antigen more prone to apoptosis. Furthermore, CD117+ T cells are highly sensitive to apoptosis mediated by galectin-1, a molecule commonly expressed within the tumour microenvironment and may therefore represent a novel, and potentially targetable, mechanism of tumour immune evasion.
**P.B2.07. Environmental regulation anti-tumor responses - Part 7**

**P.B2.07.01**

**Promoting NK cells anti-viral and anti-tumor function by in vitro manipulation**

E. Badami, F. Barberà, A. Galló, C. Coronelí, D. Poiani, P. Conaldi

1Fondazione IRCCS, Policlinico, Italy, 2ISMETT, Policlinico, Italy.

**Background/Methods.** Natural Killer (NK) cells respond to infection and tumor by releasing pro-inflammatory cytokines (IFNγ, TNFα) or by cell-to-cell contact using TRAIL-mediated apoptosis. The aim of this study was to discover if response to viral infection or cancer cells can be improved by manipulating NK cells in vitro with selected cytokines.

**Results.** Healthy donor NK cells were differentiated with a selected mix of cytokines into cytotoxic TRAIL- or cytokine-releasing IFNγ/TNFα NK cells and phenotype assessed by Flow Cytometry. Cytotoxicity was determined by CRA. NK immune function was studied in transwell co-cultures with target cells +/- HCV; miRNome signature was investigated by NGS and cytokine profiling by Multiplex.

**Conclusions.** We propose that AML cells may alter NK cell functions and maturation by secreting TGF-β. Our preliminary results are in line with observations made in human patients and will be discussed. Further experiments are needed to evaluate the potential of NK cell manipulation as a new therapeutic strategy in acute myeloid leukemia.

**P.B2.07.02**

**Underlying mechanisms of tumor recurrence after incomplete cancer immunotherapy**


Leiden University Medical Center, Leiden, Netherlands.

Cancer vaccines aim to induce specific T cell responses directed against tumor cells. Previously, we have shown therapeutic efficacy of vaccination with synthetic long peptide (SLP) vaccines against established tumors in mice and patients with human papilloma virus-induced neoplastic lesions. However, under less optimal vaccine conditions the SLP-vaccinated mice display tumor recurrence despite the initially T-cell mediated regression induced by the vaccine. Here, we investigated the underlying mechanisms focusing on the tumor microenvironment. Our data showed that recurrent tumors displayed a significantly reduced leukocyte infiltration in particular CD8 T cells compared to primary tumors. This was accompanied by lower levels of chemokine receptors including CXCR3 on the T cells, and a reduced chemokines production by intratumoral immune cells. Moreover, although these T cells are capable of producing inflammatory cytokines, the cytotoxic capacity to kill tumor cells was inferior compared to the T cells at the time of regression. Notably, we did not observe any difference in the expression of T cell inhibitory molecules such as PD-1, Tim3 and LAG3 at the time of relapse, confirming our results that blocking PD-1 did not prevent the tumor recurrence. Interestingly, vaccination of mice that were injected with recurrent tumor cells did not result in tumor regression. Together, that the observed differences between T cells during tumor growth and increasing intrinsic tumor resistance to killing occurs that incomplete cancer immunotherapy leads to immune selection and tumor escape. On-going work exploring immune selection and tumor heterogeneity by using RNA seq analysis and cell barcoding will be discussed.

**P.B2.07.03**

**A regulatory macrophage phenotype induced by IgG4: implications for tumour-mediated immune tolerance**

R. Bianchini, P. B2.07.03

**Background/Aims.** Natural Killer (NK) cells respond to infection and tumor by releasing pro-inflammatory cytokines (IFNγ, TNFα) or by cell-to-cell contact using TRAIL-mediated apoptosis. The aim of this study was to discover if response to viral infection or cancer cells can be improved by manipulating NK cells in vitro with selected cytokines.

**Methods.** Healthy donor NK cells were differentiated with a selected mix of cytokines into cytotoxic TRAIL- or cytokine-releasing IFNγ/TNFα NK cells and phenotype assessed by Flow Cytometry. Cytotoxicity was determined by CRA. NK immune function was studied in transwell co-cultures with target cells +/- HCV; miRNome signature was investigated by NGS and cytokine profiling by Multiplex.

**Results.** NK cell differentiation with any of the cytokine cocktails tested induced upregulation of NKGr2, NKp30, NKp44 and NKp46. By cell-cell contact, cytotoxic TRAIL-NK cells killed with the highest efficacy target cells, while cytokine-releasing IFNγ/TNFα NK cells were less functional. By transwell co-culture, we observed that TRAIL NK cells fully eradicated HCV infection and reduced the tumor phenotype by releasing soluble factors, while IFNγ/TNFα-NK cells were less efficient. By comparative miRNome analysis we underpinned a number of novel miRNA. Notably, hierarchical clustering showed systematic variations in the miRNA expression among the different groups. Protein analysis produced pattern of soluble factors and will be here discussed.

**Conclusions.** The pathways identified in activated TRAIL NK cells that specifically characterize their enhanced anti-viral and anti-tumor function compared to IFNγ/TNFα-NK described herein might represent a tool to license fully functional NK cells with translational potential for clinical applications.

**P.B2.07.04**

**Underlying mechanisms of tumor recurrence after incomplete cancer immunotherapy**

E. Badami, F. Barberà, A. Galló, C. Coronelí, D. Poiani, P. Conaldi

1Fondazione IRCCS, Policlinico, Italy, 2ISMETT, Policlinico, Italy.

**Background/Methods.** Natural Killer (NK) cells respond to infection and tumor by releasing pro-inflammatory cytokines (IFNγ, TNFα) or by cell-to-cell contact using TRAIL-mediated apoptosis. The aim of this study was to discover if response to viral infection or cancer cells can be improved by manipulating NK cells in vitro with selected cytokines.

**Results.** Healthy donor NK cells were differentiated with a selected mix of cytokines into cytotoxic TRAIL- or cytokine-releasing IFNγ/TNFα NK cells and phenotype assessed by Flow Cytometry. Cytotoxicity was determined by CRA. NK immune function was studied in transwell co-cultures with target cells +/- HCV; miRNome signature was investigated by NGS and cytokine profiling by Multiplex.

**Conclusions.** Underlying mechanisms of tumor recurrence after incomplete cancer immunotherapy described herein might represent a tool to license fully functional NK cells with translational potential for clinical applications.

**P.B2.07.05**

**Red pulp macrophages in the spleen provide a niche for chronic myeloid leukemia stem cells**

E. D. Bührer, M. A. Amrein, C. Nombela-Arrieta, D. Paini, P. Conaldi

1Department of Biomedical Research, Bern, Switzerland, 2Department of Hematology, Zürich, Switzerland, 3Baxter laboratory for stem cell biology, Stanford, United States, 4Department of Medical Oncology, Bern, Switzerland.

Chronic myeloid leukemia (CML) is a malignant myeloproliferative disorder characterized by the constitutively active tyrosine kinase BCR/ABL1. Disease progression and relapse is caused by therapy resistant leukemia stem cells (LSCs), and cures on their reliance on the microenvironment. The microenvironment in the bone marrow (BM) has been extensively studied and is known to contribute to LSC maintenance and resistance. Leukemic infiltration of the spleen is a hallmark of CML. However, the detailed composition of the splenic niche in CML and how it affects and maintains LSCs is unknown. In a mouse model of CML, we demonstrated that primitive leukemic stem and progenitor cells (LSPCs) preferentially accumulated in the spleen and contributed to disease progression by increasing LSC numbers in the BM. RNA sequencing of spleen and BM LSPCs revealed enriched stromas and decreased myeloid lineage priming in LSPCs of the spleen. Moreover, LSCs in the spleen were more quiescent than in LSCs in the BM and showed increased resistance to tyrosine kinase inhibitor (TKI) therapy. Furthermore, spleen LSCs were exclusively located in the red pulp. Depletion of macrophages by clodronate treatment reduced LSC numbers in the spleen, whereas LSCs in the BM were not affected. In spleens of human CML patients, LSCs co-localized with red pulp macrophages (RPMs). These results reveal the spleen as an independent, disease-promoting niche for primitive LSCs. Thus, targeting the splenic niche may be necessary to eradicate LSCs and cure CML.
POSTER PRESENTATIONS

P.B2.07.06
MHC class I modulation by iron metabolism and NK cells recognition
E. Carbone
1,2, R. Sottile
1,4, G. Federico
1, C. Garofalo
1, R. Tallarico
1, C. Faniello
1, B. Quaresima
1, C. Cristiani
1, G. Cuda
1, V. Venturini
1,2, N. Perrotti
2, S. Ferrone
2, E. Gulletta
2, K. Kärre
2, F. Carlomagno
2
1Tumor Immunology and Immunopathology Laboratory, Department of Experimental and Clinical Medicine, University “Magnna Graecia” of Catanzaro, Catanzaro, Italy, catanzaro, Italy, 2Department of Microbiology, Cell and Tumor Biology (MTC), Karolinska Institutet, Stockholm, Sweden, Stockholm, Sweden, 3Tumor immunology and Immunopathology Laboratory, University “Magnna Graecia” of Catanzaro, Catanzaro, Italy, catanzaro, Italy, 4Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 17177, Stockholm, Sweden., Stockholm, Sweden, 5Department of Molecular Medicine and Medical Biotechnologies Federico II University, Naples, Italy, Naples, Italy, 6Centro de Investigacion de Investigacion y de Bioenimie, Avanzada, Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi “Magnna Graecia”, Catanzaro, Italy, 7Dipartimento di Medicina Sperimentale e Clinica, catanzaro, italy, 8Department of Health Sciences, University “Magnna Graecia” of Catanzaro, Catanzaro, Italy, catanzaro, Italy, 9University of Medical Genetics, Mater Domini University Hospital, Catanzaro, Italy, 10Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA, Boston, United States, 11Department of Health Sciences, University “Magnna Graecia” of Catanzaro, Catanzaro, Italy, catanzaro, Italy.

Introduction: The iron concentration in the environment is crucial element for both pathogens and innate immunity. We investigate whether extracellular iron concentrations and intracellular ferritin heavy chain (FHC) may modulate MHC expression and NK cells cytotoxicity. This new and interesting link between iron metabolism and immunity can be exploited in the treatment of infections and cancer.

Materials and Methods: Primary melanoma tumor cells were treated with DFO and IFN gamma and established tumor cell lines were silenced with shHIF vector. PBMC derived from hemochromatosis patients’ blood were isolated. All cell system were analysed for the expression of MHC molecules and NK cytotoxicity assays. To analyze ex vivo and in vivo the relationship between FHC and MHC class I expression the NCO4 -/- mice was used.

Results: HFC downregulation, either by environmental iron chelation or shRNA transfection, led to MHC-class-I surface reduction. Moreover, low concentrations of iron in microenvironment interfere with IFN-gamma receptor signaling preventing the related increase of MHC-class-I molecules expression. Furthermore, ex vivo evidences confirm the in vitro observations: a) mouse bearing a NCO4 -/- gene deletion leads to FHC accumulation and MHC class I cell surface overexpression b) Hemochromatosis patients with high iron blood concentrations express higher levels of MHC-I on their cell surface. Tumor cells growing in low iron environment are highly susceptible to NK cytotoxicity.

Conclusions: We propose a role for H-ferritin and iron metabolism in regulating the MHC class I expression in humans and mice and the related NK susceptibility.

P.B2.07.07
Blocking of beta adrenergic signalling improves the efficacy of anti-tumor responses
C. Dohel
1, L. Vimeux
2, E. Peranzoni
2, R. Stoewer
2, E. Donnadieu
1, A. Trautmann
1, N. Bercovici
1, V. Feuilliet
1, Institut Cochin, Inserm U1016 CNRS UMR 1014, Université Paris Descartes, Paris, France.

Background: Adrenergic signals are known to exert a major influence in cancer. In addition to a direct action on tumor growth, numerous arguments support potential effects on anti-tumor immune responses. Our goal was to characterize the effects of adrenergic signals on anti-tumor responses, and to estimate the therapeutic interest of β-blockers for improving them.

Material and methods: To address this question, we used two murine tumor models: 1) a model of vaccine-induced regression of transplanted tumors (TC1) and 2) a model of progressive spontaneous mammary tumors (MMTV-PyMT) in which the "natural" anti-tumor response is in check. In both models, we evaluated the effects of a chronic treatment with propranolol (a β-blocker) using multicolour flow cytometry, gene expression and imaging.

Results: In TC1 model, propranolol strongly improved the vaccine efficiency by increasing the number of CD8+ T cells infiltrating the tumor. Moreover, we demonstrate that this effect may be also occurred during the priming of T cells in the draining lymph node (Dr-LN). In MMTV-PyMT tumors, tumor growth was markedly slowed down by propranolol, which also increased tumor infiltration by CD8+ T cells. Finally, in vitro, adrenergic signalling had a major inhibitory effect on T cell activation, a phenomenon likely to alter T cell reactivity in both Dr-LN and the tumor.

Conclusions: Our results allow a better understanding of the influence of adrenergic signalling on anti-tumor responses. They provide a basis for the strategic use of β-blockers to improve them.

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P.B2.07.08
Multiparameter flow cytometry immunophenotypic identification and characterization of tumor infiltrating immune cells in glioblastoma multiforme
M. González-Tablas
1,2, Á. Otero
1, D. Pascual
1, L. Ruiz
1, D. Miranda
1, P. Sousa
1, D. Arranz
1, M. Jaramillo
1, J. Gonzalvez
1, A. Orfao
1, M. Taberner
1,2,3
1Centro de Investigación del Cáncer (CIC-BIOMEC), ISCIII, and Departamento de Medicina Universidad de Salamanca, Salamanca, Spain, 2Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain, 3Servicio de Neurocirugía, Hospital Universitario de Salamanca, Salamanca, Spain, 4Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL), Soria, Spain.

Glioblastoma multiforme (GBM) is the most common and aggressive adult primary tumor of the brain. The specific distribution and role of tumor immune cells in favoring or blocking malignant transformation, tumor progression and growth, remains unknown. Here we characterize the cellular composition of tumor immune infiltrates from primary GBM by multiparametric flow cytometry.

Resected primary tumors from 39 adults diagnosed with GBM were analyzed. Single tumor cell suspensions were obtained by mechanical disaggregation and stained with 8-color monoclonal antibody combinations for the enumeration of tumor infiltrating myeloid (CD45, HLA-DR, CD14, CD11B, CD16, CD15, CD33, CD192 and CD206) and lymphoid cells (CD3, CD4, CD8, CD19, CD20, CD25 and CD127). Stained samples were measured in a Fortessa X20 flow cytometer and analyzed with the Infinicyt software.

Overall, tumor infiltrating immune cells (TICs) represented (median) 27% of the whole cellularity, with highly-variable numbers among distinct tumors (range: 3%-73%). Myeloid cells, with monocytic/dendritic cell phenotype predominated (23%; range: 2%-65%) with a minor proportion of neutrophils (2%; range: 0.2%-45%) and lymphoid cells (1.4%; range: 0.4%-8%). Among lymphocytes, CD8+ (0.5%; range: 0.02%-3%) and CD4+ T cells (0.4%; range: 0.01%-3%) were represented at similar low values, and extremely low numbers of regulatory T-cells (median: 0.05%) and B-lymphocytes (0.02%) were also found. We demonstrate the feasibility of evaluating TICs by flow cytometry and their systematic presence in GBM tumor samples at highly variable levels, with clear predominance of antigen-presenting myeloid, neutrophils and lymphoid infiltrates, their role in modulating the tumor microenvironment, deserves further investigations. [ISCIIL ref PI16/0476]

P.B2.07.09
MHC II-dependent activation of regulatory T cells in the bone marrow of leukemia mice leads to immune evasion and disease progression
M. Hinterbrandner
1, A. Ochsenbein
1, C. Riether
1, Bern University Hospital and Department for BioMedical Research, University of Bern, Bern, Switzerland.

Leukemia stem cells (LSCs) in the bone marrow (BM) are the origin of leukemia and resistant against conventional therapies and immune control. This resistance is partially mediated by protective mechanisms of the hematopoietic stem niche in the BM. In leukemia, the BM microenvironment changes dramatically with regulatory T cells (Tregs) accumulating. However, little is known how Tregs affect LSCs.

We induced chronic myeloid leukemia (CML) in a murine model with B/L6 BCR-ABL1-transduced LSKs (lineage Sca-1- c-kit+) in FoxP3+ mice. We investigated the frequency, activation, proliferation and frequency of BM Tregs in CML compared to naive mice and analyzed the Treg-accumulation during disease progression.

BM Tregs in CML mice were mostly thymic-derived, activated and showed higher proliferation capacity compared to controls phenotypically (FACS-analysis) and functionally by colony forming assays and secondary transplantation experiments. To show a direct Treg-LSC interaction or an indirect via CDB T cells, we depleted Tregs and CDB T cells simultaneously. Parallel depletion restored LSC numbers, suggesting that Tregs protect LSCs from CD8-mediated elimination. To investigate the activation of Tregs, we induced CML derived from MHC-II-deficient LSCs since we observed high MHC II expression on LSCs. MHC II+ CML developed significantly slower than control CML and showed the same phenotype as the Treg-depleted CML mice.

Our data indicate that thymic-derived, MHC-activated Tregs protect LSCs from elimination by cytotoxic CDB T cells and promote leukemia development.

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POSTER PRESENTATIONS

P.B2.07.10
Cytochalasin-related cytokines improve natural killer cell activity against cancer cells
K. A. Holder, J. Lajoie, M. D. Grant

1Memorial University, St. John’s, Canada, 2University of Manitoba, Winnipeg, Canada.

Through an unknown mechanism, cytochalasins (CMF) focuses the initially diverse natural killer (NK) cell repertoire into functionally and phenotypically distinct subsets. Given the global prevalence of CMF infection, at least 2 billion people have an adapted pool from within which NK cells can be selectively recruited against virus-infected or aberrant cells. We investigated whether cytokines produced in CMF infection alter NK cell activity against transformed cell lines. Supernatants from CMF-infected (CMFv) fibroblasts caused a 40% and 32% rise in NK cytotoxicity (K562) and antibody-dependent cellular cytotoxicity (ADCC; anti-MHC-I coated CIR-827), respectively, enhancing NK activity through NKGC2, NKGD2, Nkp30, and Nkp46. While CMvsn had elevated levels of IL-6, IL-8, IL-15, IFN-α2 and IFN-β, disrupting stimulation through IFN-α/β receptors alone fully prevented increased NK cell activity. The CMF-encoded hil-10 homolog (cmw10) also enhanced NK cytotoxicity and we probed NK activity against a variety of malignant cell lines to study the breadth of cytokine-induced NK cell activation. CMvsn or cmw10-10 treatment robustly increased NK cytotoxicity against breast (SKBR-3), ovarian (SKOV-3), prostate (22Rv1) and colon (HT-29) cancers, and myeloid (U937) cells. Exogenous cmw10-10 also induced potent TNF-α and IFN-γ responses by NK cells from CMFv donor against antibody-coated SKOV-3 cells.

P.B2.07.11
Studying TGF-β1 activation via GARP in megakaryocytes and its potential involvement in myeloproliferative neoplasms.
S. Lecomte

de Duve Institute, Brussels, Belgium.

Myeloproliferative neoplasms (MPN) represent clonal proliferation of pathological hematopoietic stem cells. MPN encompass chronic myeloid leukaemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). MPN subtypes differ in their potential to develop bone marrow fibrosis, with PMF exhibiting the highest and ET having the lowest risk of progression to bone marrow fibrosis. The precise mechanisms leading to increased deposition of bone marrow stromal fibres remain unclear. A growing body of evidence suggests that it is mediated by Transforming Growth Factor-beta 1 (TGF-β1) released by proliferating megakaryocytes. TGF-β1 is a well-known pro-fibrotic cytokine. It is secreted by many cell types as an inactive form, called latent TGF-β1. However, very few cell types have been shown to produce the active form of the cytokine, via mechanisms that are cell-type specific. Regulation of TGF-β1 expression and activity is complex, and involves a number of cell types that can activate TGF-β1 via GARP, a transmembrane protein that is induced on the surface after T cell receptor stimulation. GARP is also expressed on megakaryocytes. We want to address the question whether megakaryocytes can also activate TGF-β1 via GARP, in physiological conditions, but more importantly in the context of myeloproliferative neoplasms where this mechanism could contribute to bone marrow fibrosis.

P.B2.07.12
Receptor expression and cytotoxicity of primary human NK cells is impaired in obesity
W. Naoukis, A. Hauflé, J. Spielbergmann, I. Böhri, D. Quandt, J. Hartl, H. Kielstein

1Department of Anatomy and Cell Biology, Faculty of Medicine, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany, 2Transfusion Medicine Unit, University Hospital, Halle (Saale), Germany.

Introduction: Overweight and obesity are growing epidemic health problems. Obesity, as a major risk factor for developing severe cancers, e.g. colorectal and postmenopausal breast cancer, is associated with alterations in NK cell function. In the early phase of cancer development, NK cells are the central active component of a host’s immune system. To date the pathophysiological mechanisms between obesity and cancer remain unclear. Therefore, the present study aimed to investigate the relation between bodyweight and NK cell receptor activation against tumor cells in vitro.

Methods: PBMCs were collected from healthy donors with different body mass indexes (normal-weight, overweight, obese) and NK cell specific parameters were analyzed by flow cytometry. The cytolytic activity of isolated NK cells against human colon and breast cancer cell lines was analyzed by an impedance-based cytotoxicity assay.

Results: Primary human NK cells isolated from obese donors showed decreased cytotoxic activity compared to normal-weight donors.

Conclusions: The decreased cytotoxic activity of tumor cells correlates with the altered receptor expression pattern of NK cells from overweight and obese individuals indicating that the link between obesity and the known increased risk for colorectal cancer can be related to an impaired NK cell functionality.

P.B2.07.13
Conformational epitope PAINS-13 on the CD9 tetraspanin is expressed on clonal plasma cells in a subset of patients with monoclonal gammopathies.
A. Roncancio-Clevia, C. Martín-Martín, E. Rodríguez-Martín, P. Walo, J. Fernández-Velasco, M. Espiño, M. Blanchard, L. Villar, E. Roldán

Hospital Universitario Ramón y Cajal, Madrid, Spain.

Monoclonal gammopathies (MG) are characterized by clonal proliferation of plasma cells (PC). Several molecules have been implicated in the adhesive interactions between PC and the bone marrow (BM) microenvironment, particularly beta 1 integrin (CD29), which is sometimes associated with tetraspanins. We described that clonal PC from the majority of MG patients express a functionally conformational epitope (PAINS-13) of the tetraspanin CD9. The study included diagnosed MG patients (41 MM, 14 MGUS). BM PCs were also stained with conjugated CD38, CD56, CD19 (to detect normal and clonal PC) and CD29, CD49d, CD49f or CD9 mAb to study the expression of adhesion molecules and major histocompatibility complex (MHC) class I and II molecules or siRNA into macrophages and (ii) to subsequently release the functional cargo in an adequate amount. We developed destabilized liposomes being sensitive to low pH molecules or siRNA into macrophages and (ii) to subsequently release the functional cargo in an adequate amount. We developed destabilized liposomes being sensitive to low pH

P.B2.07.14
Destabilized liposomes as carriers for doxorubicin and siRNA to target tumor associated macrophages in a humanized mouse melanoma model
J. Schupp, M. Voigt, M. Helm, A. Tuettenberg

1Dept. of Dermatology, Mainz, Germany, 2Johannes Gutenberg University, Mainz, Germany.

An established tumor did overcame the patient’s immune system and is exploiting its immune suppression mediating mechanisms to sustain tumor growth and avoid rejection. Resolving tumor associated macrophages into immunoinsulator M1 macrophages is a promising strategy to flip the switch in the tumor microenvironment from immune suppression towards an immune active tumor. In order to achieve repolarization of macrophages it is necessary to have (i) efficient nano carriers to transport small molecules or siRNA into macrophages and (ii) to subsequently release the functional cargo in an adequate amount. We developed destabilized liposomes being sensitive to low pH levels and physiological temperatures. The chemotherapeutic Doxorubicin and siRNA are used as cargo. We perform release studies in vitro and in vivo.

To analyze cargo release we perform in vitro cultures of human monocyte-derived macrophages and human melanoma cells. Doxorubicin release inside the cell is quantified by using flow cytometry and confocal microscopy. siRNA mediated transfection is detected via qPCR. In vivo studies are carried out in a subcutaneous melanoma model of human melanoma cell lines in humanized NOD/SCID mice transgenic for HLA-A2. Spleen and tumor showed a distinct composition of immune cell infiltration in immunohistochemistry staining proving the model to be valid for our purpose. Our data indicate that destabilized liposomes can elicit faster release of doxorubicin compared to stable liposomes. Control liposomes loaded without cargo are non-toxic. Therefore, we use them as nano carrier in our model. This work is supported by the DFG (CRC1066).
L. E. Vannucci, F. Cajo1, D. Stakeeva1, O. Chernyavskiy, P. Trenti2, P. Lukaš, L. Rajagov1, T. Hudcov1, R. Stepankova, H. Kozakova, J. Dvořák, J. Krizan1, P. Sima3, R. Makovicky3, R. Sedlacek1,2, P. Makovicky1, 2, Institute of Microbiology of the CAS, v.v.i., Prague 4, Czech Republic, 1Faculty of Science, Charles University, Prague, Czech Republic, 2Institute of Physiology of the CAS, v.v.i., Prague 4, Czech Republic, 3Institute of Molecular Genetics of the CAS, v.v.i., Prague 4, Czech Republic, 4Czech Centre for Phenogenomics, Vestec, Czech Republic, 5Selye Janus University, Komarno, Slovakia.

Conventional (CV) and germ-free (GF) animals offer a potent model to understand the complex interplay between tissue and immune system structure for modeling, e.g. in the colon. The constant immune activation sustained by colon microbiota can be put in comparison with immune activation of the GF animal nais mucosal system variously induced (bacteria, dextran sodium sulfate, azoxymethane). After induction, the bowel was harvested at established time points. Samples were taken for histology and second-harmonic generation (SHG) analysis by multi-photon confocal microscopy. Mucosa samples were analyzed for cytokine expression (ELISA, PCR). The healthy CV rat revealed higher pro-inflammatory than TAMs isolated post radiation. While these two populations share similar proinflammatory features, they differ in their response to treatment. Our preliminary data show similar activation of both macrophage subpopulations, with a shift toward an M2-like phenotype in the early onset stages of treatment, and acquisition of a common phenotype at recurrence. However, these phenotypes differ in intensity, indicating that despite a similar differentiation, GM and MG respond differently to irradiation. We now aim to further understand the molecular mechanisms underlying BMDD and MG influence on GBM recurrence. These research questions will shed light on the heterotypic communication between glioma cells and their local and systemic environment.

The inflammatory threshold guides tissue remodelling: the example of germ-free animal conventionalization and induced colitis

The inflammatory threshold guides tissue remodelling: the example of germ-free animal conventionalization and induced colitis

Macrophage subpopulations acquire distinct education programming along radiation treatment response in gliomas

Tumor-associated macrophages in rectal cancer polarize to the proinflammatory M1 phenotype after irradiation in patients and 3D co-culture model

Tumor-associated macrophages in rectal cancer polarize to the proinflammatory M1 phenotype after irradiation in patients and 3D co-culture model

Dissecting the role of MS4A4A in the context of macrophages

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Oxidative stress in the microenvironment of B cell chronic lymphocytic leukemia

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Tumor-associated macrophages initiate anti-tumoral (M1) or immunosuppressive (M2) responses depending on their polarization status. To test the effects of radiotherapy, we ex vivo irradiated tissue samples of human rectal cancer and assessed the phenotype of macrophages, T cells and NK cells by flow cytometry. We evaluated their distribution after short course radiotherapy (n=45) and compared findings to non-pretreated rectal cancer (n=25) using an immunostaining approach. We further investigated the influence of cancer-associated fibroblasts and cancer cells on the polarization of macrophages after ex vivo irradiation using 3D co-culture models. Irradiated rectal cancer samples contained less CD68+ macrophages (18.2±12.6 vs 63.6±87 total leukocytes) with a viability of >92% in both groups. Stainings of markers associated with the M1 (CD64, CCR7, ΝΟx, TNFa, HLA-DR, CD86) or M2-like (CD206, CD163, IL-10, IL-4) phenotype revealed an increase of M1/M2 ratio arguing for a shift from M2- to M1-like macrophages due to irradiation. Irradiated tissue sections demonstrated diminished T cell counts (109.7±8.68 vs 45.71±7.26 CD3+ cells/mm²) but elevated infiltration of NK cells (50.3±15.51 vs 75.91±4.3 CD56+ cells/mm²). The polarization of 3D co-culture models led to a dose dependent increase of M1/M2. Untreated macrophages in co-cultures without fibroblasts tended to be M2 but more M1-like. Neutralizing IL-10 antibody induced M1-like macrophages. Treatment with recombinant IL-10 partly rescued the effects of irradiation. Our findings highlight macrophage effectors as crucial effectors on Aspergillus fumigatus infection, having a key role in the infection context. Thus, we are characterizing the susceptibility of ms4a4a-/- mice to A. fumigatus infection, a major risk factor in immunocompromised patients. A better understanding of MS4A4A expression and function in the context of macrophages will pave the way to the use of this molecule as a therapeutic target or prognosis marker in both tumor and infection context.

Multiple markers have been reported to identify specific MØs subsets and activation profiles. Recently, we described the tetraspanin MS4A4A as part of the transcription signature of M2-MØs and tumor-associated MØs (TAMs). It was shown that MS4A4A is selectively expressed in tissue resident MØs, is upregulated during polarization towards an M2/M2-like phenotype, being also highly expressed in TAMs. The pattern recognition receptor Dectin-1 was identified as a molecular partner of MS4A4A. MS4A4A-deficient MØs present an impaired Dectin-1 dependent crosstalk with NK cells, leading to uncontrolled metastatic spreading. Yet, several aspects of MS4A4A immunobiology remain unclear. Our goal is to better understand the functional consequences of MS4A4A interaction with Dectin-1 and other partners. We are taking advantage of genetic models to characterize MS4A4A expression in the mouse. A second line of work concerns the characterization of MS4A4A role in carcinogenesis and metastatic spreading, with several models being currently under investigation. Dectin-1 recognizes β-glucans in Aspergillus fumigatus cell wall, having a key role in the infection context. Thus, we are characterizing the susceptibility of ms4a4a-/- mice to A. fumigatus infection, a major risk factor in immunocompromised patients. A better understanding of MS4A4A expression and function in the context of macrophages will pave the way to the use of this molecule as a therapeutic target or prognosis marker in both tumor and infection context.

A better understanding of MS4A4A expression and function in the context of macrophages will pave the way to the use of this molecule as a therapeutic target or prognosis marker in both tumor and infection context.
**Abstracts of the 5**

**POSTER PRESENTATIONS**


1Molecular and Translational Immunology Laboratory, Department of Clinical Biochemistry and Immunology, Universidad de Concepción, Concepción, Chile. 2Unidad de Anatomía Patológica, Hospital Las Hijueras, Talcahuano, Chile. 3IMRC Centre for Transplantation, School of Immunology & Microbial Sciences, King’s College London, Guy’s Hospital, SE1 9RT, London, United Kingdom. 4Unidad de Anatomía Patológica, Hospital Guillermo Grant Bavenoite, Concepción, Chile. 5Departamento de Ingeniería Informática y Ciencias de la Computación, Facultad de Ingeniería, Universidad de Concepción, Concepción, Chile.

Regulatory T-cells (Tregs) are a subset of CD4+ T-cells that maintain immunological tolerance and regulate immune homeostasis. Tregs have been classified as regulatory T helper (Th) cells according to the expression of specific transcription factors, cytokines and chemokine receptors that mirror effector Th lineages. We have recently characterised peripheral blood and tissue resident Th-like Tregs in healthy donors and patients with cancer. Our results revealed thatCCR4-expressing Th2-like Tregs were enriched in tumorigenic areas compared to healthy tissues. However, it is not clear whether Tregs migrate in response to specific chemokines or whether the environment supports the differentiation of Th2-type Tregs. Chemotactic assays demonstrated that Th2-like Tregs migrate preferentially to chemokines CCL17 and CCL22. However, CCR8, a chemokine receptor associated with tumour-infiltrating Tregs, was also preferentially expressed in Th2-like Tregs, therefore it is also possible that Th2-like Tregs migrate in response to CCL1 and CCL2. In this study, we evaluated the expression of CCL1, CCL17, CCL18 and CCL22 in cancer tissues from patients with oral squamous cell carcinoma compared to healthy oral mucosa. Our results demonstrated that patients with oral cancer expressed higher levels of CCL18 and CCL17. These results suggest that both, CCR4 and CCR8, can support the migration of Th2-like Tregs to malignant areas in oral cancer.

**P.B.3.01 T-cell regulation - Part 1**

**P.B.3.01.01 Abundance of Tregs and effects of their inhibition in oral cancer**

S. Aggarwal, S. Sharma, S. Das; All India Institute of Medical Sciences, Delhi, India.

Oral squamous cell carcinoma (OSCC) is one of the major cancers affecting in Asian countries. The main causative factor has been tobacco habit. It has been reported that immune dysfunction in these patients is one of the major factors for tumor growth and dissemination that affects disease-free survival of the patients. We assessed the phenotypic and functional characteristics of Regulatory T (Treg) cells in OSCC patients by multicoloured flow cytometry.

Using flow cytometry analyses of cells from 46 cancer patients, we observed a positive correlation between CCR3 expression on Treg and tumor tissue compared to unaffected colon tissue, and also observed a positive correlation between CCR3 expression among intratumoral Treg and CCR3 expression among circulating Treg (p < 0.01). Furthermore, in vivo, tumor-infiltrating Treg express significantly more CCR3 and Foxp3 on a per cell basis, as well as markers indicating increased turnover and suppressive function, such as K67, ICOS, PD-L1 and CTLA-4. Functional suppression assays suggest potent suppressive capacity of CD39+ Treg on proliferation and IFN-γ secretion by conventional T cells. Preliminary studies also indicate that high levels of CCR3 expression among intratumoral Treg may correlate to a worse patient outcome. In conclusion, our results show a large infiltration of CD39+ Treg in colon tumors, and this subset appear more immunosuppressive than their CD39 counterparts. We suggest that immunotherapy aimed at reducing tumor-infiltrating CD39+ Treg activity may be particularly useful in the setting of colon cancer.

Funding sources: Swedish Research Council and Swedish Cancer Foundation.

**P.B.3.01.02 CD39+ regulatory T cells accumulate in colon adenocarcinoma and display markers of increased suppressive function**


Increasing knowledge of the function and regulation of tumor-infiltrating lymphocytes has led to new insights in cancer immunotherapy. Regulatory T-cells (Treg) accumulate in colon tumors, and we recently showed that CD39 Treg from cancer patients inhibit transendothelial migration of conventional T-cells. CD39 mediates the hydrolysis of ATP to immunosuppressive adenosine and adds to the immunosuppressive effects of Treg. Here, we further investigated the regulatory features of intratumoral CD39+ Treg in colon cancer.

Using flow cytometry analyses of cells from 46 cancer patients, we confirmed the accumulation of CD39+ Treg in the tumor tissue compared to unaffected colon tissue, and also observed a positive correlation between CD39 expression among intratumoral Treg and CCR3 expression among circulating Treg (p < 0.01). Furthermore, tumor-infiltrating Treg express significantly more CCR3 and Foxp3 on a per cell basis, as well as markers indicating increased turnover and suppressive function, such as K67, ICOS, PD-L1 and CTLA-4. Functional suppression assays suggest potent suppressive capacity of CD39+ Treg on proliferation and IFN-γ secretion by conventional T cells. Preliminary studies also indicate that high levels of CD39 expression among intratumoral Treg may correlate to a worse patient outcome. In conclusion, our results show a large infiltration of CD39+ Treg in colon tumors, and this subset appear more immunosuppressive than their CD39 counterparts. We suggest that immunotherapy aimed at reducing tumor-infiltrating CD39+ Treg activity may be particularly useful in the setting of colon cancer.

Funding sources: Swedish Research Council and Swedish Cancer Foundation.

**P.B.3.01.03 Lymphopenia induces homeostatic T-cell proliferation after autologous stem cell transplantation**


Homotypic hematopoietic stem cell transplantation (HCT), an increasingly common treatment for many types of cancer and immune disorders, comes at a cost of lymphopenia and the concomitant need for immune reconstitution. A successful reconstitution depends on the early recovery of T lymphocytes. However, T-cell recovery after SCT generally occurs extremely slowly. There is evidence in mice that lymphocyte production and/or survival rates increase when cell numbers are low. Studies in humans have suggested that increased T-cell proliferation after SCT is associated with clinical events (GVHD) rather than with low lymphocyte numbers. Here, we investigated whether T-cell production is increased after SCT patients were treated with thymocyte and splenocyte culture systems.

In vivo, deuterium labelling and mathematical modelling we quantified the dynamics of T-cells in patients who underwent autologous HCT, and had no signs of infectious complications. After a reconstitution period of up to 1.5 years, absolute numbers of CD4+ T-cells, particularly of the naive subset, were still very low in these patients. Deuterium labelling demonstrated that the production rates of naive and memory CD4+ and CD8+ T-cells in SCT patients were significantly increased compared to healthy individuals. TREC content analysis of CD31 expression of naive CD4+ T-cells suggested that these increases in T-cell production were due to increased peripheral proliferation, and not thymic output. Taken together, this work shows that despite the slow reconstitution of lymphocyte numbers after SCT in humans, lymphocyte proliferation rates are in fact homeostatically increased in response to lymphopenia, bringing new insights in the cellular dynamics behind a timely T-cell reconstitution.

**P.B.3.01.04 Tyrosine kinase inhibitor Dasatinib effects on INKT cells and innate CD8 T-cells in chronic myeloid leukemia patients**

A. Barbarin', L. Lefere', M. Abdollahi, N. Piccirilli, E. Capossi', L. Roy, A. Herbelin', J. M. Gambert'1,2,4; 1INSERM U1082, Poitiers, France. 2CHU de Poitiers, Poitiers, France. 3Hôpital Henri Mondor, Créteil, France. 4Université de Poitiers, Poitiers, France.

Introduction: Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell malignancy caused by the presence of the chimerical BCR-ABL oncoprotein, with deregulated tyrosine kinase (TK) activity. Dasatinib is a highly potent second-generation BCR-ABL tyrosine kinase inhibitor (TKI) with off-target immunological effects. We recently identified in healthy individuals a distinct new innate CD8 T-cell subset characterized by the expression of NK receptors (KIR/NGK2A2), high Eomesodermin expression and prompt IFN-γ production, Fasciolus de Ingenia. Universidad de Concepción, Concepción, Chile.

Posters

**P.B.07.20 Characterization of human T helper-like regulatory T cells and chemokine expression in oral squamous cell carcinoma**


1Molecular and Translational Immunology Laboratory, Department of Clinical Biochemistry and Immunology, Universidad de Concepción, Concepción, Chile. 2Unidad de Anatomía Patológica, Hospital Las Hijueras, Talcahuano, Chile. 3IMRC Centre for Transplantation, School of Immunology & Microbial Sciences, King’s College London, Guy’s Hospital, SE1 9RT, London, United Kingdom. 4Unidad de Anatomía Patológica, Hospital Guillermo Grant Bavenoite, Concepción, Chile. 5Departamento de Ingeniería Informática y Ciencias de la Computación, Facultad de Ingeniería, Universidad de Concepción, Concepción, Chile.

Analysis showed an increase of iNKT cells with a Th1 profile. Despite the decrease of the memory CD8 T-cell compartment, the innate-memory CD8 T-cell pool was increased.

Results: In CML patients treated 12 months with Dasatinib, we observed an increased percentage of both iNKT cells and innate CD8 T-cells. In the mouse model, in vivo and in vitro analysis showed an increase of iNKT cells with a Th1 profile. Despite the decrease of the memory CD8 T-cell compartment, the innate-memory CD8 T-cell pool was increased. Conclusion: Taken together, our data are in favor of a direct effect of Dasatinib on both iNKT cells and innate CD8 T-cells numbers and functions.
POSTER PRESENTATIONS

P.B3.01.05
Unconventional T-\gamma cells (iNKt and innate CD8 T cells) in solid tumors and their tumor environment
A. Barbarini1, B. Moreira1, N. Piccini1,2, G. Nazarre Luna2, E. Piaggio2, V. Lavoué2, V. Catara3, A. Herbelin4, J. M. Gombert4;
1INSERM U1082, Poitiers, France, 2CHU de Poitiers, Poitiers, France, 3CIBM 1428 SIRIC, Institut Curie, Paris, France, 4CHU de Rennes, INSERM U991, CRN de Rennes, Rennes, France,
5Université de Poitiers, Poitiers, France.

Introduction: Cancer immuno-surveillance involves innate and adaptive cells as well as non-conventional T cells like iNKt cells. Recently, we have identified in humans a new CD8 T cell subset, named innate CD8 T-cells, expressing a classical TCR-\alpha\beta and NK markers and responding to innate-like stimulation by the pro-inflammatory cytokines IL-12, IL-18 and IL-33. Our recent data suggest a possible link between iNKt cells and innate CD8 T-cells via the secretion of IL-4 by iNKt cells. Here, our aim was to study the iNKt/innate CD8 T cell axis in solid tumors.

Methods: We analyzed by flow cytometry blood samples, ascites, pleural fluid, carcinosarcoma and tumor samples from patients with ovarian, breast, pancreas or colon cancer for the presence and activation state of iNKt cells and innate CD8 T-cells. IL-12, IL-18 and IL-33 levels were measured in plasma and ascite supernatants by ELISA.

Results: iNKt cells and innate CD8 T-cells were present in the tumor, carcinosarcoma and ascites. iNKt cells from the tumor and tumor environment were enriched in CD69 positive cells, reflecting their higher activation state than their peripheral counterparts. Furthermore, we observed a positive correlation between the expression of the two transcription factors characterizing iNKt and innate CD8 T-cells, PLZF and Eomes, respectively. Finally, we showed that the tumor environment was enriched in IL-12 and IL-33 whereas IL-18 was detected at the periphery.

Conclusions: Taken together, our results support the hypothesis of anti-tumoral cooperation between iNKt cells and innate CD8 T-cells in response to the cytokines IL-12 and IL-33 in solid tumors.

P.B3.01.06
Regulatory T-cell compartment in HIV+ pregnancies loses gestation adaptations and function
A. Cockr1, S. Sivarajasingam1, A. Sassine2, I. Raj3, S. Dermont4, A. Khan2, N. Imam1, M. Johnson2;
1Imperial College London, London, United Kingdom, 2Chelsea and Westminster Hospital, London, United Kingdom.

Regulatory T cells (Treg) are thought to maintain tolerance towards the fetus during pregnancy, and mediate responses to viruses such as Cytomegalovirus (CMV) that risk disrupting immunological balance. HIV+ women have higher incidences of preterm labour, potentially caused by increased immune activation. Here the longitudinal development of the Treg compartment and its relation to viral replication in HIV-1+ and HIV-1+ pregnancies is described.

Peripheral blood mononuclear cells were isolated from pregnant HIV-1+ (n=19) and HIV-1- (n=15) women, and flow cytometric analysis performed to determine the frequency of Treg cells (CD3+CD4+CD25+CD127low) and their expression of CD45RA, and HLA-DR. In HIV+ and HIV-1+ women, Arl4d was PD-L1 dependently highly induced in naïve CD8 T cells and that Arl4d functions to repress their production of IL-2. As PD-L1/PD-1 signaling may inhibit effector function of CD8 T cells, the discovery of Arl4d and PD-L1 may result in a positive relationship in the HIV+ women.

The Treg compartment demonstrates gestational changes and correlates to anti-viral responses, supporting their suppressive function. Loss of these relationships in HIV+1+ participants highlights this compartment as clinically relevant to their increased preterm labour rate.

P.B3.01.07
Role of the interleukin-33/ST2L axis for the CD8-dependent anti-cancer cytotoxicity
C. Dreis1, F. Ottenlinger1, M. Herrera San Juan2, M. Putryska1, G. Ernst1, M. U. Martin1, J. Pfeilschifter1, H. H. Radeke1;
1pharmazentrum Frankfurt, Frankfurt am Main, Germany, 2Institute of Biochemistry II, Frankfurt am Main, Germany, 3Immunology F808 Justus-Liebig-University, Giessen, Germany.

Novel cancer therapies target the activation of the tumor antigen specific cytotoxic T cells to improve treatment efficacy. For the alarmin interleukin-33 (IL-33), ligand of the Th2 marker ST2L and family member, increasing evidence suggests an involvement in Th1 immunity. We previously demonstrated IL-33/IL-33 dependent co-activation of murine cytotoxic T cells and hypothesize induction of anti-tumoral Th1 activity by IL-33. We re-evaluated the regulation of IL-33 bioactivity within optimized in vitro bioactivity assays and analyzed regulation of ST2L in ST2 expression of human immune cells.

IL-33 aa (amino acid) 111-270 and recombinantly generated hypertrophic aa295-270 potently activated ST2L expressing HEK293 reporter cells. In a competitive assay with ST2L, significant downregulation of IL-33 bioactivity required 100 fold excess of soluble decoy receptor sST2. However, in the absence of ST2L, aa111-270 and aa295-270 exhibited high binding affinities to sST2 (2.21 nM ± 1.1; 13.47 ± 2.9 nm). IL-33 and IL-1B detected by ELISA in serum of healthy donors (n=30) failed to induce a corresponding bioactivity. Still, bioactivity of exogenous recombinant IL-33, but not IL-1B, was significantly reduced in human plasma. Proteases and oxidation have been excluded as elicitors of this effect.

Peripheral blood mononuclear cells and isolated CD8+ T cells revealed expression of cell surface ST2L as well as sST2 mRNA. These results support our hypothesis of a crucial role of the interleukin-33/ST2L-axis for the CD8-dependent anti-cancer cytotoxicity.

P.B3.01.08
The role of the PD-1 inducible ARF-like GTPase 4D in immune inhibition of anti-tumor CD8 T cell immunity
B. Geers1, P. Sprezynja, J. Lind, E. Diehl;
1University Medical Center Hamburg-Eppendorf, Institute of Experimental Immunology and Hepatology, Hamburg, Germany.

Blockade of the inhibitory PD-L1/PD-1 pathway holds promise for cancer immunotherapy. Priming of CD8 T cells by liver sinusoidal endothelial cells (LSEC) leads to development of the tumor environment. By the increasing evidence suggests an involvement in Th1 immunity. We previously demonstrated IL-33/L-3/L-dependent co-activation of murine cytotoxic T cells and hypothesize induction of anti-tumoral Th1 activity by IL-33. We re-evaluated the regulation of IL-33 bioactivity within optimized in vitro bioactivity assays and analyzed regulation of ST2L in ST2 expression of human immune cells.

IL-33 aa (amino acid) 111-270 and recombinantly generated hypertrophic aa295-270 potently activated ST2L expressing HEK293 reporter cells. In a competitive assay with ST2L, significant downregulation of IL-33 bioactivity required 100 fold excess of soluble decoy receptor sST2. However, in the absence of ST2L, aa111-270 and aa295-270 exhibited high binding affinities to sST2 (2.21 nM ± 1.1; 13.47 ± 2.9 nm). IL-33 and IL-1B detected by ELISA in serum of healthy donors (n=30) failed to induce a corresponding bioactivity. Still, bioactivity of exogenous recombinant IL-33, but not IL-1B, was significantly reduced in human plasma. Proteases and oxidation have been excluded as elicitors of this effect.

Peripheral blood mononuclear cells and isolated CD8+ T cells revealed expression of cell surface ST2L as well as sST2 mRNA. These results support our hypothesis of a crucial role of the interleukin-33/ST2L-axis for the CD8-dependent anti-cancer cytotoxicity.

P.B3.01.09
Evaluating in vivo anti-tumor T cell responses to melanoma using newly developed tumor cell lines
J. L. Hope1, M. L. Barrosa, R. Tinoce, I. M. Bradley;
1Sanford Burnham Prebys Medical Discovery Institute, La Jolla, United States.

Mouse models of cancer remain the preferred means to assess the efficacy of anti-cancer therapies, and several models have been used to address the effect of cancer drugs or in the discovery of biomarkers. The YUMM1.5 and YUMMER1.7 murine tumor models were developed from the Braf/Pten engineered mouse model to more closely reflect melanomas that are human disease-relevant models; however, the dominant T cell-recognized epitopes specific for these tumors remains unknown. We therefore sought to engineer OVA-expressing versions of the YUMM1.5 and YUMMER1.7 tumor lines, hereafter referred to as YUMM1.5-OVA and YUMMER1.7-OVA. Using stable transfection of mice expressing full-length secretory OVA, we have generated polyclonal and monoclonal cell lines that generate in vivo OVA-specific response as validated by MHC class I tetramer staining and cytokine production following OVA peptide stimulation. Together, these two novel models for melanoma will allow us to assess intrinsic and extrinsic mechanisms regulating the generation of effective and recovery of exhausted T cell responses to melanoma in vivo.

P.B3.01.10
Immunogenicity and immunophenotypes in young-onset colorectal cancers
M. E. Jisselstijn1, T. Brouwer2, D. Roano1, R. van der Breggen3, H. Morreau4, K. Jordanova5, N. De Miranda1;
1Leiden university medical centre, Leiden, Netherlands.

Immunotherapy has emerged as one of the most promising options for cancer treatment. Tumor-infiltrating immune cells have great prognostic value in solid tumors, including colorectal cancer. In recent years, a tendency has been observed towards an increased incidence of colorectal cancer in young (<50) patients. These patients are not included in screening programs and thus often diagnosed at advanced stages of tumor progression. To our knowledge, no study has yet characterized immunophenotypes and immune evasive mechanisms in young-onset colorectal cancers. To that end, we investigated the expression of HLA class I and PD-L1 by Immunohistochemistry in over 200 young-onset colorectal cancers. Furthermore, we applied a novel multiplexed immunofluorescence technique to simultaneously assess T cell-related markers.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 275
We describe that HLA class I is maintained in 62% of mismatch-repair proficient tumours, which allows the development of neo-antigen targeted therapies, but lost in 78% of mismatch-repair deficient tumours. Interestingly, the epitopes are specifically observed in liver metastasis, which suggests a specific pressure that could be utilised for immunotherapeutic exploitation. As previously demonstrated, PD-L1 expression is limited and often restricted to immune cell compartments. Multispectral immunofluorescence imaging allows for a comprehensive overview of T-cell immunophenotypes and their relation to HLA class I expression in young-onset colorectal cancer. Retained HLA class I expression in the majority of colorectal cancers associated with low infiltration by effector immune cells suggests the possibility for therapeutic induction of immune responses in young-onset colorectal cancers, for instance by anti-neo-antigen targeted therapies.

Pos. B3.01.11

GITR targeting enhances functionality of tumor-infiltrating T cells in hepatocellular carcinoma


1Erasmus MC - University Medical Centre, Rotterdam, Netherlands; 2Vrije Universiteit, Brussels, Belgium; 3Pfizer inc., South San Francisco, United States.

No curative treatment options are available for advanced hepatocellular carcinoma (HCC). A recent study demonstrated that anti-PD1 antibody therapy can induce tumor regression in 20% of advanced HCC patients, thereby revealing that co-inhibitory immune checkpoint blockade may have therapeutic potential for this type of cancer. However, whether agonistic targeting of co-stimulatory molecules, e.g., hexameric or trimeric TNF-related apoptosis inducing ligand (TRAIL), can induce regression of larger tumor in HCC is as yet unknown. We studied expression of the co-inhibitory receptor GITR in tumor-infiltrating lymphocytes (TIL) isolated from freshly resected tumors, and on lymphocytes isolated from paired tumor-free liver tissues and blood of HCC patients. We determined whether agonistic targeting of GITR could enhance ex vivo functional responses of HCC TIL. In all three tissues, GITR was predominantly expressed on CD4FOXP3+ regulatory T cells (Treg). The highest expression levels were found on CD4FOXP3+CD25RA activated Tregs in tumors. Addition of recombinant GITR-ligand or a humanized agonistic antibody against GITR (DH918, Pfizer) to cultures of HCC TIL enhanced CD8+ T cell proliferation, granyme B and IFNγ production, in response to OX40/CD28 stimulation. GITR ligation also enhanced proliferative responses of tumor-derived CD4+ and CD8+ TIL to tumor antigens presented by mRNA-transfected autologous 8 cells blasts. Interestingly, GITR-targeting expressing Treg, CD4FOXP3+ T helper cells, and CD8+ T cells in tumors co-expressed PD-1. Combining GITR ligation with anti-PD1 antibody further enhanced proliferative responses of CD4+ and CD8+ TIL to tumor antigens compared to either single treatment. Conclusion: Agonistic targeting of GITR may be a promising strategy for single or combined immunotherapy in HCC.

Pos. B3.01.12

Thymus derived T cell development is regulated by T cell type mediated BIC/miRNA155 expression

R. Sánchez Díaz1, R. Blanco Domínguez2, S. Lasarte3, K. Tölögön2, E. Martin Gaya2, B. Llimillas Pradilla4, H. de la Fuente5, S. Sánchez Madrid1, R. Nakajima2, M. Toribío1, P. Martini6

1CINC, Madrid, Spain, 2Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, Madrid, Spain; 3Hospital de la Princesa, Madrid, Spain; 4The Francis Crick Institute, London, United Kingdom; 5CIBER de Enfermedades Cardiovasculares, Instituto de Salud Carlos III, Madrid, Spain.

Background and OBJECTIVE: Thymus-derived regulatory T cells (Treg) are key to preventing autoimmune diseases, but the mechanisms involved in their development remain unsolved. Here, we show that the C-type lectin receptor CD69 controls treg development and peripheral Treg cell homeostasis through the regulation of BIC/miRNA155 (miR-155) and its target, suppressor of cytokine signaling 1 (SOCS-1).

Methods: Using Foxp3miR155cd69Fop or Foxp3miR155cd69Fop reporter mice and short hairpin RNA (shRNA)-mediated silencing and miR-155 transfection approaches, we found that CD69 deficiency impaired the signal transducer and activator of transcription 5 (STAT5) pathway in Foxp3+ cells. Results: miR-155 inhibition, increased in CD69+/-tigreg cell development in embryos, and Rag2-/- y v- hematopoietic chimeras reconstituted with cd69-/- stem cells. Accordingly, mirn155+/- mice have an impaired development of CD69+ treg cells and overexpression of the miR-155-induced CD69 pathway, suggesting that both molecules might be concomitantly activated in a positive-feedback loop. Moreover, in vitro-inducible CD25+ Treg (tigreg) cell development is inhibited in if239+cd69- mice.

Conclusions: Our data highlight the contribution of the CD69 as a nonredundant key regulator of BIC/miR-155-dependent Treg cell development and homeostasis.

Pos. B3.01.13

GMP-compliant generation of human granzyme B regulatory B cells for the therapy of graft-versus-host disease

C. Menga1, A. Felsen1, L. Kurz2, T. Trzaska2, P. Reinhardt2, H. Schrzenmeier2, D. Fabricius2, K. Schilbach2, B. Jähnsdörfer3

1Department of Transfusion Medicine, Ulm, Germany; 2Institute for Clinical Transfusion Medicine and Immunogenetics, German Red Cross Blood Transfusion Service Baden-Württemberg – Hessen and University Hospital Ulm, Ulm, Germany; 3Department of Pediatrics, Ulm, Germany; 4Department of General Pediatrics, Oncology/Hematology, Eberhard-Karls University Tübingen, Tübingen, Germany.

Granzyme B (GrB)-secreting regulatory B cells (Breg) suppress T cell proliferation by GrB-mediated degradation of the T cell receptor and are involved in various pathologies. Their exploitation as novel cell-therapeutic agents may therefore represent a promising approach for the treatment of graft-versus-host disease (GVHD). Recently, we developed a cocktail consisting of IL-21 and antibodies against the human B cell receptor, allowing for easy ex-vivo induction of GrB+ Breg from peripheral B cells isolated from whole blood. In our current study, we now used a GMP-compliant positive selection kit to directly isolate CD19+ B cells from leukapheresis products collected from eight unstimulated healthy donors. Subsequently, we tested the isolated B cells in terms of their potential to differentiate into GrB+ Breg. On average, we were able to isolate 56.5±0.8Breg cells from “6±10” total PBNB. PBNB varied from 0.1% to 1.3%, indicating a generation of ~600 Vpre-B cells. After 48 hours of incubation, an average of 64.7±0.8 cells showed the typical GrB+ phenotype. Of note, these B cells maintained their GrB+ phenotype for up to another 72 hours after the end of incubation. In conclusion, our findings demonstrate that GMP-compliant generation of induced Breg is feasible. Our results pave the way for further development of Breg as novel cell-therapeutic agent. A first pilot study on the effect of GrB+ Breg on GVHD in a humanized mouse model will be starting in June 2018. Initial results will be discussed on the conference.

Pos. B3.01.14

MicroRNA-33 is induced in repeatedly activated T Helper 1 lymphocytes and regulates their motility

M. Bardou1, C. Hoffmann1, P. Durek1, M. Magraith1, C. Tran2, G. Heins3, P. Maschmeyer1, M. Lohoff1, H. Chang4, A. Radbruch1, M. Mascherbi

1Deutsches Rheuma-Forschungszentrum, Berlin, Germany; 2Institute for Medical Microbiology and Hygiene, University of Marburg, Marburg, Germany.

T helper (Th) lymphocytes can be readily found in inflamed tissues of patients with rheumatic diseases despite immunosuppressive therapies and express the transcription factor Twist1, which is a functional biomarker for Th1 cells with a history of repeated (auto)jungentic stimulation. Little is known about the molecular adaptations which allow these cells to persist in the inflamed tissues. To mimic Th cells from either protective immune reactions or chronic inflammation, we have generated acutely (once) and repeatedly (four times) activated Th cells in vitro. By performing high throughput screening of small RNAs, we have identified the microRNA-33 (miR-33) being selectively upregulated in repeatedly activated murine Th1 (Th1 rep) and in memory Th1 lymphocytes isolated from the splenic fluid of patients suffering from rheumatic joint diseases as compared to once activated Th1 cells or memory Th cells isolated from the blood, respectively. In order to identify direct targets and the molecular function of miR-33, we determined the transcriptomes of Th1 rep cells after antagomir mediated miR-33 knockdown. We could not identify a role of miR-33 in the suppression of actin cytoskeleton in these cells but lacking miR-33 expression, in an in vitro transwell migration assay Th1 rep cells showed 50% less migration as compared to once activated Th1 cells. This reduction in the migratory capacity is partly caused by miR-33 knockdown in Th1 rep cells. MiR-33 might represent a molecular switch important for the persistence of proinflammatory Th1 cells in inflamed tissues.

Pos. B3.01.15

Characterising immune dysregulation in the inflammatory skin disease Hidradenitis suppurativa

B. Morari1, J. Musilova1, R. Hughes2, A. Malard2, K. H. Mills3, D. C. Winter1, A. Tobin1, B. Kirby1, J. M. Fletcher1

1Trinity College Dublin, Dublin, Ireland; 2St Vincent’s University Hospital, Dublin, Ireland; 3Tallaght Hospital, Dublin, Ireland.

Introduction: Hidradenitis suppurativa (HS) is a chronic, debilitating skin disease with 1.4%-prevalence and high morbidity. Symptoms include painful lesions, which leak a bloody, suppurative, foul-smelling discharge. These lesions can merge to form dermal tunnels, leading to restricted and painful movement, and a significant reduction in quality of life. Risk factors include obesity and smoking, with a 3:1 female:male ratio. HS pathogenesis is poorly understood, with immune dysregulation strongly implicated. Materials and Methods: Cells isolated from skin biopsies and blood from HS patients and healthy volunteers were dissociated and analysed by flow cytometry to characterise infiltrating cells, and reveal their function. A fraction of blood was reserved for immunohistochemistry analysis. Results: We observed substantial immune cell infiltration in HS lesions, including significantly increased numbers of neutrophils, macrophages, B, and Th17 T cells. Particularly, T cells, particularly CD8 T cells, and macrophages were recruited to HS lesions. Further, HS lesions appeared to carry a much less inflammatory phenotype, similar to that of healthy individuals. Conclusion: These data suggest that IL-17 inhibition via TNF blockade is a promising approach for the treatment of immune dysregulation in HS, and provides a rationale for targeting the IL-17 pathway in the disease.
Regulatory T cells constitute a significant immunosuppressive factor in BCR-ABL-positive chronic myeloid leukemia (CML), as they participate in inhibition of effector immune response against leukemic cells. As CML cells are autologous and upregulate various self-antigens, thymic Treg (tTreg), as auto-tolerant cells, are of significant interest. Immune cells can be regulated by extracellular vesicles (EVs) secreted by cancer cells, as demonstrated in solid tumors. Leukemic EVs have been shown to influence stromal and endothelial cells in bone marrow niche, but their immunomodulatory role has not been explored. We have investigated role of leukemic EVs in differentiation of Treg, using different mouse models, including ex vivo model of Treg differentiation and in vitro suppression assay, performed by multicolor flow cytometry. EVs from 32D BCR-ABL+ cells were isolated by differential ultracentrifugation and characterized by TEM, nanoparticle tracking and western blotting. Mature, sorted tTreg exposed to CML-derived EVs exhibit higher suppressive activity. They also express significantly higher level of Foxp3 transcription factor, which suggests global regulation of tTreg function. This effect is attenuated by BCR-ABL inhibitor (imatinib) treatment. We also show that even though CML-derived EVs do not increase Treg differentiation ex vivo, naïve thymocytes exposed to these EVs during ex vivo culture and eventually differentiated into Tregs demonstrate higher level of Foxp3 and higher suppressive activity. Collectively, our results demonstrate that leukemic extracellular vesicles upregulate suppressive activity of both differentiating and mature Treg, thus suggesting a novel immunosuppressive mechanism in chronic myeloid leukemia.

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1University Health Network, Toronto, Canada
2University of Toronto, Toronto, Canada, Hospital for Sick Children Research Institute, Toronto, Canada
3Laboratory T (Reg) cells expressing the transcription factor FOXP3 are essential for the maintenance of immunological self-tolerance but play a detrimental role in most cancers due to their ability to suppress antitumor immunity. The phenotype of human circulating Treg cells has been extensively studied, but less is known about tumor-infiltrating Treg cells. We studied the phenotype and function of tumor-infiltrating Treg cells in ovarian cancer and melanoma to reveal potential Treg cell-associated molecules that can be targeted by tumor immunotherapies. Treg cells isolated from ovarian tumors displayed a distinct cell surface phenotype with increased expression of a number of receptors associated with TCR engagement, including PD1, 4-1BB and ICOS. Higher PD-1 and 4-1BB expression was associated with increased responsiveness to further TCR stimulation and increased suppressive capacity, respectively. Transcriptomic and mass cytometry analyses revealed the presence of Treg cell subsets and further supported a highly activated state specifically in ovarian tumors. Moreover, Treg cells infiltrating melanomas displayed lower FOXP3, PD-1, 4-1BB and ICOS expression and were not as potent suppressors of CD8 T cell proliferation. The highly activated phenotype of ovarian tumor-infiltrating Treg cells may be a key factor in the establishment of an immunosuppressive tumor microenvironment and constitute a roadblock to successful immunotherapy. Receptors that are specifically expressed by tumor-infiltrating regulatory T cells could be exploited for the design of novel combination tumor immunotherapies.

R. Casetti, V. Bordoni, G. Grassi, A. Agrati

3National Institute for Infectious Diseases Lazzaro Spallanzani, Rome, Italy.

A. Sacchi, N. Tumino, A. Sabatini, E. Cimini, R. Casseti, V. Bordoni, G. Grassi, A. Agrati

National Institute for Infectious Diseases Lazzaro Spallanzani, Rome, Italy.

Mature, sorted tTreg exposed to CML-derived EVs exhibit higher suppressive activity. They also express significantly higher level of Foxp3 transcription factor, which suggests global regulation of tTreg function and constitute a roadblock to successful immunotherapy. Receptors that are specifically expressed by tumor-infiltrating regulatory T cells could be exploited for the design of novel combination tumor immunotherapies.


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R. Casetti, V. Bordoni, G. Grassi, A. Agrati

3National Institute for Infectious Diseases Lazzaro Spallanzani, Rome, Italy.

P. S. Ohashi, P. A. Shaw

P. A. Shaw

P. S. Ohashi, P. A. Shaw
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.B3.02.04
Identification of a key epigenetic barrier towards terminal cytotoxic T lymphocyte differentiation
1 The Netherlands Cancer Institute, Amsterdam, Netherlands, 2 VU University Medical Center, Amsterdam, Netherlands

T cell development and differentiation requires highly specific and tight epigenetic regulation, involving specific alterations to the chromatin structure by covalent modifications of histones. Here we study the role of histone modification, specifically H3K79 methylation in T cell biology. H3K79 methylation is a unique histone mark that requires the activity of the evolutionary conserved methyltransferase DOT1L. H3K79me is associated with transcription and in some cases with the inhibition of repression of the genes it methylates. To assess whether this histone mark plays a role in the terminal effector stage, DOT1L ablation did not grossly affect intrathymic T cell development. However, transcriptome analysis of single positive (SP) mature CD8 T cells revealed an upregulation of memory associated genes that normally require antigen exposure in the periphery. This effect of DOT1L ablation was most striking in the peripheral CD8+ T cells. Here, without any deliberate immunogenic exposure, the virtual absence of naive CD8 T cells was accompanied with a dramatic increase in memory-like CD8+ T cells. Our results indicate that DOT1L is a key element in establishing a barrier towards terminal T cell differentiation. Future studies will address the functional potential of T cells lacking DOT1L as well as the mechanism by which the methylation mark at H3K79 maintains naïveté.

P.B3.02.05
Immunomodulatory effects of a soluble form of human CD6 in experimental cancer
S. Casadi-Llombart*, I. T. Simões, E. Velasco-de Andrés, M. Consuegra-Fernández, F. Aranda*, V. Carreras, F. Lozano*1,2
1,2 Institute of Investigaciones Biomédicas August Pi i Sunyer, Barcelona, Spain, 3 Servei d’Immunologia, Centre de Diagnòstic Biomèdic, Hospital Clínico de Barcelona, Barcelona, Spain, 4 Departament de Biomedicina, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain

CD6 is a surface receptor expressed by all T cells and a subset of B and NK cells. In T cells, it is physically associated to the TCR/CD3 complex and it mediates cell adhesion and modulation of T cell signaling during T cell activation and differentiation, likely through interactions with its reported ligands CD166/ALCAM, CD318 and Galectin-1/3. To elucidate the in vivo relevance of CD6-mediated interactions, the decoy receptor effectors of recombiant soluble human CD6 (rshCD6) were analyzed. Thus, CD7/8- mice treated with rshCD6 (1.25mg/kg i.p.) every other day showed significantly reduced total cell numbers in spleen, increased total cell numbers in peritoneum and decreased percentage of Treg in lymph nodes, compared with vehicle-treated control mice. The functional significance of such response was evidenced by observation of an enhanced anti-tumour response when rshCD6-treated mice were simultaneously challenged with melanoma B16-F0 cells (7x10^3 cells i.c.) compared with human seraalbumin-treated controls. This work demonstrates that CD6 is upregulated in infiltrating T cells and significantly increases the proportion of Treg cells in tumour-draining and contralateral lymph nodes. In vitro studies showed that rshCD6 decreased Treg but not Th1, Th2 and Th17 polarization of naive CD4+ T cells in a dose-dependent manner. Taken together, these results support decoy CD6 ligand-receptor interactions by rshCD6 as a feasible strategy for immunomodulation in cancer. Supported by WCR (14-1275), Fundacio La Marató TV3 (201319-30), Portuguese FCT (SFRH/BD/75738/2011, and Spanish MINECO [SAF2016-80535-R, BES-2014-069237]), ISCIII (SB Program; CD15/00016), and MECD (FPU15/02897), -co-financed by European Development Regional Fund "A way to achieve Europe".

P.B3.02.06
Improved survival in HPV oropharyngeal cancer patients is associated with tumour-resident CD8+ T cells
R. V. Hewavasanthi1, A. L. Ferguson, D. Jones1, A. Hong, U. Palendira*
1,2 Centenary Institute for Cancer Medicine and Cell Biology, Sydney, Australia, 3 Central Clinical School, Royal Prince Alfred Hospital, Sydney, Australia.

Introduction: Human papilloma virus-positive (HPV+) oropharyngeal squamous cell carcinoma (OSCC) is a clinically and immunologically distinct subset of head and neck cancer, which is increasing in prevalence. HPV+ tumours have shown improved patient prognoses compared to HPV-negative (HPV-) tumours. Tumour-resident CD8+ T cells (TR8s) have been associated with better patient survival in various cancers, but have not been extensively studied in virally-induced cancers. Differing patient outcomes, between HPV+ and HPV- tumours, illustrate the importance of tailoring treatments to specific subsets of OSCC, which may be altered in intensity based on immunological mechanisms. To understand the role of TR8s in HPV-OSCCs, we investigated whether TR8 numbers correlate with patient survival, in the context of HPV status, tumour microenvironment, and clinical outcomes. Materials and Methods: Infiltrating TR8s were analysed by quantitative multiplex immunofluorescence staining and multi-parameter flow cytometry, using tumours from a prospective cohort of OSCC and HPV-OSCC patients. Results: HPV-OSCC patients had higher TR8 numbers compared to HPV+OSSC patients, with higher infiltration of total CD8+ T cells within HPV+ tumours. Increased TR8 numbers were associated with better patient prognosis and improved survival in HPV+OSCC patients, compared to those with lower TR8 numbers. Conclusions: The higher TR8 numbers found in HPV+OSSC, compared to HPV-OSCC, suggests that TR8s may be critical in controlling OSCC tumour progression, resulting in better patient prognosis. Further investigation of TR8s may provide a better understanding of how we approach vaccine strategies, with the aim of boosting TR8 numbers and improving the immune response.

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P.B3.02.07
TCR/ITK signaling tunes CD8+ T cell metabolism, homeostatic proliferation and anti-tumor effector function
W. Huang*, A. Luo, A. August1,2
1 Department of Pathological Sciences, Louisiana State University, Baton Rouge, LA, United States, 2 Department of Microbiology and Immunology, Cornell University, Ithaca, NY, United States, 3 Department of Biological Chemistry and Neuroscience, Center for Sensory Biology, The Johns Hopkins University School of Medicine, Baltimore, MD, United States, 4 Howard Hughes Medical Institute, Cornell University, Ithaca, NY, United States.

T cell homeostatic proliferation is regulated by T cell receptor (TCR) signals and homeostatic cytokines, and suggested to be proportional to TCR signal strength. However, we show that ITK, a positive regulator of TCR signaling, negatively tunes CD8+ T cell metabolism, proliferation and effector function. Under lymphopenic environments, ITK-/- CD8+ T cells exhibit T-cell intrinsic, immediate and massive HR, which requires ITK for efficient homeostatic proliferation and effector function in the periphery. This effect of ITK on T-cell-mediated CD8+ T cell metabolism and HR is an mTOR-dependent manner. The lack of ITK also resulted in enhanced effector cell programming, antigen sensitivity and anti-tumor immunity of the HP cells. Thus TCR signaling via ITK, is a negative tuner of CD8+ T cell homeostasis, metabolism and effector function, and may be a target for clinical benefit in cancer therapy.

P.B3.02.08
BTLA and PD-1 signaling pathways regulate proliferation and cytotoxicity of human V delta 2 gamma/delta T cells independently
H. Huang, K. Koh*, J. Lee*, S. Kang*, H. Im*, N. Kim*
1,2 Asan Institute for Life Sciences and Department of Convergence Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of; 1 Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of; 2 Department of Pediatrics, Korea University Anam Hospital, Seoul, Korea, Republic of.

B- and T-lymphocyte attenuator (BTLA) and programmed cell death-1 (PD-1) inhibit T cell activation and function through recruitment of SHP2. Although there are studies on the individual roles of BTLA and PD-1 in human gammadelta T cells, it is not well known how these immune checkpoints interact in response to cancer. This study was set up to examine whether BTLA and PD-1 signaling pathways were convergent or independent in human peripheral blood gammadelta T cells, where Vgammadelta T cells recognizing phospho-antigens are majority. Herein we show that BTLA/HEV2 and PD-1/PD-L1 interactions suppressed proliferation and cytotoxicity of human gammadelta T cells, respectively. As expected, IL-2 and zinc-finger co-induction of proliferated gammadelta T cells were co-cultured with inactivated HEV2+ Jurkat cells, compared with that of wild-type Jurkat cells. CD107a expression and cancer cell death were not affected in expanded gammadelta T cells by co-culture with inactivated HEV2+ Jurkat cells and further increased in the presence of anti-PD-1 mAb. The results suggest that inactivation of BTLA/HEV2 signaling pathway during expansion could help produce more gammadelta T cells without compromising cytotoxicity. In addition, red shift of HEV2 expression in Jurkat cells repressed phosphorylation of SHP2 and increased activation of ERK1/2 in gammadelta T cells. However, blockade of PD-1 signaling did not have a synergistic or additive effect on it. Taken together, our study demonstrates that BTLA and PD-1 signaling may independently act on the proliferation, cytotoxicity and phosphorylation of SHP2 and ERK1/2 in human Vgammadelta T cells.

P.B3.02.09
Costimulatory molecule B7-H3 in Myelodysplastic syndrome
A. Kindermann, R. Mandasari, D. Quandt
University of Halle, Halle, Germany.

Costimulation is an important way of regulating T cell immunity. B7-H3 belongs to the B7 family of costimulatory molecules and has been shown to modulate T cell outcomes in health and disease. Many solid tumor cells in vitro show a differential expression of B7-H3 that is associated to patient’s outcome. The role of B7-H3 for hematological disease is not well established. Myelodysplastic syndrome (MDS) is a hematological stem cell disease leading to diverse phenotypes of altered red and white blood cell hematopoiesis. Analyses of human bone marrow samples from patients with MDS, other hematological disorders and healthy donors using flow cytometry were performed. B7-H3 expression was found on blast cells, HSC as well as on myeloid cells. In leukaemia (THP-1, HL-60, K562) as well as MDS cell lines, B7-H3 protein is expressed constitutively with a great heterogeneity. This is in contrast to solid tumor cell lines that show a homogenous high level of B7-H3 expression.

278
Cytokine treatment with important adaptive as well as innate mediators did not alter B7-H3 protein expression, whereas other molecules of the B7 family as well as the adaptive co-stimulatory molecules are highly regulated. Co-cultures of B7-H3 modulated THP-1 cells with human primary T cells are currently performed to further understand the functional consequences of this costimulatory pathway. T cells are an important part of the stem cell niche. Therefore we believe on a contribution of costimulatory molecules on the regulation of the complex hematopoietic stem cell network.

P.B3.02.10
Strong sustained IL-2 signal selectively targeted to CD25+ cells dramatically increases sensitivity to LPS
J. Tomala, P. Weberova, B. Tomalova, M. Kovar;
Institute of Microbiology, Prague, Czech Republic.
IL-2 exerts its pleiotropic activities through binding either to dimeric receptor composed from IL-2Rα (CD122) and common cytokine receptor gamma chain (γc, CD132) or to trimeric receptor composed from IL-2Rα (CD25), IL-2Rβ and γc. CD25 has been termed "low-affinity" IL-2R (Kd~10 nM) and it is not involved in signal transduction. A dimer of CD122 and CD132 binds IL-2 with intermediate affinity (Kd~1 nM) and is present on CD122+ populations, namely memory CD8+ T cells and NK cells. A complex of CD25, CD122 and CD132 binds IL-2 with high affinity (Kd<10 pM) and it is present on CD122+ cells, namely activated T and Treg cells. It was reported that IL-2 is biologically active of IL-2 can be dramatically increased by association of IL-2 with anti-IL-2 mAbs. These IL-2 complexes possess selective stimulatory activity determined by the clone of anti-IL-2 mAbs used. IL-2/IFN-γ complexes are highly stimulatory for NK and memory CD8+ T cells and intermediate also for Treg cells. IL-2/IFN-γ complexes are stimulatory solely for CD25+ T cells. We have found that mice treated with IL-2/IFN-γ complexes dramatically showed increased sensitivity to LPS-mediated shock and mortality (~10-30 times). Mice treated with IL-2/IFN-γ complexes and challenged with 10 μg LPS possess 5-10 times higher plasma concentration of TNF-α (90 min. after LPS challenge) in comparison to control mice challenged with 200 μg LPS. Interestingly, IL-2/IFN-γ complexes almost do not sensitize mice to LPS. Acknowledgement: This work was supported by grant 13-128BSS5 from the Czech Science Foundation Research Concept RVO 61388717.

P.B3.02.11
IFN-γ and TNF-α production by T cells in HNSCC stroma promotes a distinct transcriptional signature with immunosuppressive properties by tumor-enriched mesenchymal stem cells
A. Mazzoni, G. Montani, M. Ramazzotti, G. Barra, L. Maggi, M. Capone, M. Rossi, B. Rossettini, R. De Palma, L. Cosmi, F. Liotta, F. Annunziato;
1University of Florence, florence, Italy, 2University of Campania, napoli, Italy.
Introduction: Mesenchymal stem cells (MSC) are enriched in Head-Neck squamous cell carcinoma (HNSCC) and display immunosuppressive properties, thus favoring tumor immune escape. MSC functionalities are highly regulated. Co-cultures of MSC and the molecular program of the T-cell population is gaining. Methods: IFN-γ responsive human MSC were sorted by flow cytometry. Tim-3 and c-MAF expression on T-cells were determined by immunocytochemistry. Results: The mean fluorescent intensity (MFI) of Tim-3 expression showed a non-significant increasing trend in the MLNs (P=0.091), however when considering patients with invasive ductal carcinoma (P=0.001), and for Treg cells isolated from the metastatic (MLNs) and non-metastatic lymph nodes (nMLNs). The observed Treg profile showed a non-significant increasing trend in the MLNs (P=0.091), however when considering patients with invasive ductal carcinoma (P=0.001). Moreover, we identified specific human effector Treg markers including VDR and BATF, showing striking overlap with tumor-infiltrating Treg. Our data demonstrate that human inflammation-derived Treg acquire a specific effector Treg profile guided by epigenetic changes, including VDR and BATF. Conclusions: We identified specific human effector Treg markers including VDR and BATF, showing an overlap with tumor-infiltrating Treg. Our data demonstrate that human inflammation-derived Treg acquire a specific effector Treg profile guided by epigenetic changes, including VDR and BATF. Moreover, we identified specific human effector Treg markers including VDR and BATF, showing an overlap with tumor-infiltrating Treg. Our data demonstrate that human inflammation-derived Treg acquire a specific effector Treg profile guided by epigenetic changes, including VDR and BATF.

P.B3.02.12
Tim-3+CD4+ T cells in the breast tumor draining lymph nodes
F. Mehdipour, S. Sharifat, A. Ghods, A. Tokei, A. Ghaderi; 1Shiraz Institute for Cancer Research, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of; 2Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of.
Introduction: It has been suggested that T cell immunoglobulin and mucin domain-3 (Tim-3) is an important immune checkpoint receptor which can be involved in the immune suppression in the tumor microenvironment. It has been shown that exhaustion or regulatory T cells express Tim-3. In this study, the frequency of Tim-3+CD4+ T cells in the tumor draining lymph nodes (TDLNs) of breast cancer patients and its relation with disease parameters were investigated.
Materials and methods: Using Ficoll-Hypaque gradient centrifugation, mononuclear cells were isolated from axillary lymph node specimens of 35 breast cancer patients. After surface staining for CD4 and Tim-3, cells were subjected to flow cytometry.
Results: Our results revealed that 6.4±5.8% of CD4+ T cells expressed Tim-3 without significant difference in the metastatic (MLNs) and non-metastatic lymph nodes (nMLNs). The mean fluorescent intensity (MFI) of Tim-3 expression showed a non-significant increasing trend in the MLNs (P=0.091), however when considering patients with invasive ductal carcinoma (P<0.001) and excluding patients whose tumor had medullary features, the MFI of Tim-3 was significantly higher in the MLNs (P=0.023). In addition, the MFI of Tim-3 expression had significant direct correlation with the number of involved lymph nodes (R=0.3, P=0.042). The frequency of Tim-3 expressing T cells or Tim-3 MFI did not show significant association with stage, grade or tumor size.
Conclusion: This study revealed that a fraction of CD4+ T cells expressed Tim-3 in the TDLNs of breast cancer patients. Lymph node involvement was associated with higher intensities of Tim-3 expression on T cells.

P.B3.02.13
Conserved human effector regulator T cell signature is reflected in super-enhancer landscape
1University Medical Center Utrecht, Utrecht, Netherlands, 2King’s College London, London, United Kingdom.
Regulatory T cells (Treg) are critical regulators of immune homeostasis. Increasing evidence demonstrates that environment-driven Treg differentiation into effector Treg is crucial for optimal functioning. However, programming of human Treg under promotes a distinct transcriptional signature with immunosuppressive properties by tumor-enriched mesenchymal stem cells. The observed Treg profile showed a non-significant increasing trend in the MLNs (P=0.091), however when considering patients with invasive ductal carcinoma (P<0.001) and excluding patients whose tumor had medullary features, the MFI of Tim-3 was significantly higher in the MLNs (P=0.023). In addition, the MFI of Tim-3 expression had significant direct correlation with the number of involved lymph nodes (R=0.3, P=0.042). The frequency of Tim-3 expressing T cells or Tim-3 MFI did not show significant association with stage, grade or tumor size. The observed Treg profile showed a non-significant increasing trend in the MLNs (P=0.091), however when considering patients with invasive ductal carcinoma (P<0.001) and excluding patients whose tumor had medullary features, the MFI of Tim-3 was significantly higher in the MLNs (P=0.023). In addition, the MFI of Tim-3 expression had significant direct correlation with the number of involved lymph nodes (R=0.3, P=0.042). The frequency of Tim-3 expressing T cells or Tim-3 MFI did not show significant association with stage, grade or tumor size.
Conclusion: This study revealed that a fraction of CD4+ T cells expressed Tim-3 in the TDLNs of breast cancer patients. Lymph node involvement was associated with higher intensities of Tim-3 expression on T cells.

P.B3.02.14
Type 1 regulatory cells (Tr1) express c-Maf in response to CD55 but not CD28 costimulation
T. Musarat;
University of Nottingham, Nottingham, United Kingdom.
A healthy immune system is maintained in a state of balance between pro- and anti-inflammatory cells. The paradigm for T-cell activation requires CD80/86/CD28 engagement resulting in differentiation of pro-inflammatory receptor. However, alternative costimulatory molecules may favour the induction of alternate T-cell phenotypes such as Type 1 Regulatory T cells (Tr1). One such receptor-ligand pair is CD55-CD97. These are widely expressed on leukocytes, including T cells, dendritic cells (DC) and macrophages. We have previously demonstrated that costimulation of T cells via CD55/CD28 results in the differentiation of naive T cells into Tr1 phenotype which is defined as IL-10+, IFN-γ− and as opposed to Th1 phenotype (IFN-γ+, IL-10−, IL-4−) by CD3/CD8 costimulation. IL-10 is the predominant inhibitory cytokine produced by adaptive immune system and it is required for immune resolution, promoting tolerance and controlling autoimmunity. Considering the important role of IL-10− Tr1 in immune-balance, there still remains little in the way of a defined phenotype for these cells. We aimed to study CD3/CD55 induced Tr1 cells in order to determine the transcription factors associated with the regulation of these cells. CD3/CD55 induced IL-10+ Tr1 cells were positive for T-bet and c-Maf whereas they were negative for Foxp3, GATA3 and HEVOS. Interestingly, c-Maf was only expressed by IL-10+ cells in response to CD3/CD55 but not to CD3/CD28 stimulation. c-Maf expression was persistent upon secondary restimulation with CD3/CD28 and was not induced by non-specific stimulation with PMA/ionomycin indicating that c-Maf expression could be an integral part of signalling for CD3/CD55 mediated IL-10 production by Tr1 cells.

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Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 279
Non-genomic effects of the glucocorticoids (GC) play an important role in the GC-induced apoptosis of thymocytes. We have found that in the most GC sensitive, DP thymocytes the activated GC receptor (GR) translocated to the mitochondria, which was followed by the decrease of the mitochondrial membrane potential, an early sign of apoptosis. The activated GR associated with members of the Bcl-2 protein family, especially with Bim, and influenced their balance leading to the activation of mitochondrial apoptotic pathway. The effect of in vivo dexamethasone (DX) treatment on regulatory T cells (Tregs) was different; they seemed to be resistant to DX-induced apoptosis. Which suggests that the GC-induced apoptotic process is different in other cell populations. But the exact mechanism requires further investigation. In our work we analysed the differences and similarities between the DX-induced apoptosis of thymocytes and mature T cells, especially that of Tregs. Splenocytes and thymocytes were isolated from 4-6 week-old BALB/c mice. The cells were treated with 10^5 M DX in vitro for different intervals. After DX treatments Annexin V labelling, kinetics of caspases’ activation, mitochondrial membrane potential and Ca^2+ signalling were analysed with flow cytometry. DX-induced apoptotic process was different in mature T cells, particularly in Tregs, than in thymocytes. DX inhibited Ca-signalling of cell populations suggesting its potential role in apoptotic signaling. These results can be the consequences of the different non-genomic effects in T cell subpopulations. Funding: CITKA K105962, K101493, EFOP-3.6.1-16-2016-00004; GINOP 2.3.2-15-2016-0050.

P.B3.03.17
Engineering chimeric antigen receptor T cells to specifically target Aspergillus fumigatus M. Seif1, T. Nerrer2, M. Machwerth1, M. Nedicke1, F. Ebel1, H. Einsele2, L. Löffler1
1University Hospital Würzburg, Würzburg, Germany, 2Ludwig-Maximilians-University, Munich, Germany.

Immunocompromised patients are susceptible to invasive fungal infections mainly caused by Aspergillus fumigatus (AF). Adoptive transfer of Aspergillus-specific T cells reduces the burden of invasive aspergillosis. Such specific T cells are hard to isolate and expand. Alternatively, T cells modified to express a chimeric antigen receptor (CAR) can be used. CARs are recombinant receptor constructs composed of an extracellular single-chain antibody fragment (scFv) linked to an intracellular signaling module. To redirect specificity towards AF, a scFv derived from an antibody directed against Hyphal cell wall was designed. CARs containing the scFv fused to extracellular IgG4-Fc spacer domains of different lengths were constructed. T cells were engineered to express the CARs on their surface using the Sleeping Beauty gene transfer system. CAR T cells were co-cultured with AF germ tubes and specific T cell activation was evaluated.

Upon binding to the target, CAR T cells signaled via chimeric CD28 and CD3-ζ signaling domain. The cytolytic machinery of CAR T cells was activated leading to the release of perforin and granzyme B. Activated CAR T cells secreted cytotoxic granules like ifNk-γ and IL-2. Furthermore, activated CAR T cells underwent proliferation. Finally, CARs containing long "hinge"-CD19-CH3" extracellular spacer conferred superior T cell activation when compared with CARs having short "hinge-only" spacer.

Our results show that CAR T cells are specifically activated upon recognition of AF and customizing spacer design enhances their effector function.

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P.B3.03.02
Human memory CD8 T cell effector function is epigenetically preserved during in vivo homeostasis H. Abdelham1, A. Moustaki1, Y. Fan1, P. Dogra2, H. Ghozmi1, C. Zebby2, B. Tripillet1, R. Sekaly1, B. Youngblood1
1St Jude Children’s Research Hospital, Memphis, United States, 2Case western Reserve University, Cleveland, United States.

Maintenance of memory CD8 T cell function and quality through antigen-independent homeostatic proliferation is vital for sustaining long-lived T cell-mediated immunity, yet the underlying mechanisms that preserve memory T cell function and quality during in vivo residence remain largely unexplored. Here we show that preservation of effector-potential among human memory CD8 T cells during in vitro and in vivo homeostasis is coupled to maintenance of memory-associated DNA methylation programs. Whole-genome bisulfite sequencing of primary human naive, short-lived effector memory (TM1), and long-lived central memory (TM2) CD8 T cell populations identified demethylated promoters of effector molecules that are poised for rapid expression among all memory cell subsets. Effector-loci demethylation was heritably preserved during IL-7 and IL-15 mediated in vitro cell proliferation. In contrast to the effector-potential, antigen-independent proliferation induced a phenotypic conversion of TM1 and TM2 memory into TM1cells that was coupled to increased methylation of the CRK7 locus. Furthermore, in vivo proliferation of haploidentical donor memory CD8 T cells in lymphodepleted recipients resulted in a similar preservation of effector-associated methylation programs while enriching for Tem-associated programs. These data demonstrate that long-lived human memory CD8 T cells retain the ability to undergo antigen-independent effector program reprogramming during their developmental conversion into other memory subsets while at the same time preserving the poised effector state utilized by all memory T cells. Further investigation into upstream signaling events that promote changes in T cell epigenetic states is needed to uncover the role of epigenetics in T cell function during homeostasis.

P.B3.03.01
T3 regulation - Part 3

P.B3.03.01.15
Glucocorticoid-induced apoptosis in thymocytes and mature T cells L. Prenek, R. Kugyekia, F. Balázs, P. Németh, T. Berki
Department of Immunology and Biotechnology, Pécs, Hungary.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

Non-genomic effects of the glucocorticoids (GC) play an important role in the GC-induced apoptosis of thymocytes. We have found that in the most GC sensitive, DP thymocytes the activated GC receptor (GR) translocated to the mitochondria, which was followed by the decrease of the mitochondrial membrane potential, an early sign of apoptosis. The activated GR associated with members of the Bcl-2 protein family, especially with Bim, and influenced their balance leading to the activation of mitochondrial apoptotic pathway. The effect of in vivo dexamethasone (DX) treatment on regulatory T cells (Tregs) was different; they seemed to be resistant to DX-induced apoptosis. Which suggests that the GC-induced apoptotic process is different in other cell populations. But the exact mechanism requires further investigation. In our work we analysed the differences and similarities between the DX-induced apoptosis of thymocytes and mature T cells, especially that of Tregs. Splenocytes and thymocytes were isolated from 4-6 week-old BALB/c mice. The cells were treated with 10^5 M DX in vitro for different intervals. After DX treatments Annexin V labelling, kinetics of caspases’ activation, mitochondrial membrane potential and Ca^2+ signalling were analysed with flow cytometry. DX-induced apoptotic process was different in mature T cells, particularly in Tregs, than in thymocytes. DX inhibited Ca-signalling of cell populations suggesting its potential role in apoptotic signaling. These results can be the consequences of the different non-genomic effects in T cell subpopulations. Funding: CITKA K105962, K101493, EFOP-3.6.1-16-2016-00004; GINOP 2.3.2-15-2016-0050.

P.B3.03.16
The role of Ca^2+ dependent proteins for the killing capacity of human cytotoxic T cells S. Zöphel1, G. Schward1, P. Leidinger1, K. S. Friedman1, A. Knörck1, C. Hoeh1, E. Meese1, V. Helms1, M. Haft1, M. Hamed1, E. C. Schwarz1
1Biophysics, Hamburg, Germany, 2Human Genetics, Hamburg, Germany, 3Center for Bioinformatics, Saarbrücken, Germany, 4Institute for Biostatistics and Informatics in Medicine and Biophysics, Saarbrücken, Germany.

Introduction: Cytotoxic T lymphocytes (CTLs) eliminate infected or transformed cells by releasing perforin-containing cytoxic granules at the immunological synapse. Cytotoxic granule release is highly dependent on the influx of extracellular Ca^2+, mediated by STIM-activated Orai channels. Previous work has shown that members of the SNARE-family are primarily responsible for exocytosis of lytic granules. However, considerably less is known about other molecules modulating the Ca^2+ influx and exocytosis of cytoxic granules, and thereby elimination of target cells. Material and Methods: Human PBMC were isolated from LRS chambers provided by the local blood bank. Subtype isolation was performed by antibody-coated magnetic beads. Total RNA from non-stimulated CD8 T cells or from Staphylococcus aureus enterotoxin A (SEA) stimulated CD8 T cells was isolated and investigated by microarray technology. Expression data were further analyzed by a bioinformatical approach. Results: Based on the results of the bioinformatics analysis, we selected 42 siRNAs against highly expressed proteins with a potential function in calcium signaling pathways and screened for a putative role in target cell killing. By using a real-time killing assay under reduced extracellular Ca^2+ concentrations, we identified several candidates which altered the killing capacity of CD8 T cells. One candidate is the Ca^2+- induced potassium channel KCa3.1. Its downregulation decreased the elimination of target cells under limited extracellular Ca^2+ concentration. Conclusion: Bioinformatical analysis is a powerful tool to narrow-down possible candidates relevant for target killing by CTL. Screening of the selected siRNAs by a real-time killing assay led to positive hits under limited extracellular Ca^2+ concentration.

P.B3.03.17
Tissue-resident memory T-lymphocytes (T RM) display proliferative capacity in vitro under hypoxic conditions A. Chuwonpad1, R. Stark1, F. M. Behr1, G. Schwär1, H. Abdelsamed1,2, T. H. Wesselink2,3
1Center for Bioinformatics, Saarbrücken, Germany, 2Institute for Biostatistics and Informatics in Medicine and Biophysics, Saarbrücken, Germany, 3European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands.

Tissue-resident memory T cells (T RM) have been recently established as an important subset of memory cells that provide early and essential protection against reinfection in the absence of specific adaptive immunity. However, little is known about their characteristics and their role in the context of cancer or infection. In the present work, we investigated whether T RM from the small intestine proliferate after anti-CD3/28 stimulation and subsequent resting in cytokines. The expanded T RM cells with a T RM-like phenotype have been found to infiltrate tumor tissue. The presence of these T RM-like cells is associated with patient survival, suggesting that T RM also have a protective role in cancer. Immunotherapy using adoptive transfer of in vitro reactivated T cells from the tumor site has provided a breakthrough in the treatment of cancer patients. However, this approach is hampered by the majority of patients. Because T RM are beneficial in both cancer and infection settings, we aim to study whether these memory T cells can be expanded in culture for immunotherapy-related purposes. We have found that T RM from the small intestine proliferate in vitro after anti-CD3/28 stimulation and subsequent resting in cytokines. The expanded T RM retained their phenotype, including expression of key T RM markers CD69 and CD103. Optimal culture of T RM required low O2 tension, indicating that these cells can resist hypoxic conditions in the tumor microenvironment. T RM also exerted increased metabolic activity and efficiently acquired glucose, a requirement to compete with highly glycolytic tumor cells. These findings suggest that in vitro re-stimulation of existing T RM is possible under conditions reflecting the tumor microenvironment, which is promising for future usage of these memory T cells in tumor treatment using adoptive cell therapy.
POSTER PRESENTATIONS

P.B3.03.03 Dissecting the role of MAZR isoforms in T cell development and function

M. Dhele, L. Anderson, A. Güllich, S. Sakaguchi, W. Elmenier; Medical University of Vienna, Vienna, Austria.

T cells are key players of adaptive immunity and their differentiation and function has been tightly controlled. We previously identified that the transcription factor MAZR, also known as Patz1, is an important regulator of CD8 gene expression during the double-negative (DN) to double-positive (DP) transition of T cell development. Further, by generating MAZR-deficient mice we showed that MAZR is part of the transcription factor network regulated CDA/CDB cell fate decision of DP thymocytes. MAZR is a member of the BTB domain and zinc finger (ZF) motif containing transcription factor family and is encoded by the Patz1 gene. There are 4 potential alternative splice forms known that can give rise to MAZR isoforms containing 4-7 ZF domains, however whether all splice forms are expressed in T cells has not been described. Moreover, the role of the various MAZR isoforms in the regulation of T cell development and function is not known. The aim of the study is to dissect isoform-specific functions of MAZR by using gain-of-function and loss-of-function approaches in combination with RNA-seq and ChIP-seq experiments. Results from ongoing studies will be presented. The project is funded by the EU ITN Grant “ENLIGHT-TRAIN” (675395) and the Austrian Science Fund (P29790).

P.B3.03.04 Regulation of integrin activation on antigen-specific T cells by GalpHA, -coupled receptor signaling and sleep in humans

S. Dimitrov, T. Lange, C. Gouttefangeas, A. T. Jensen, M. Szczepanski, J. Lehnensoe, S. Sekelov, H. Rammsense, J. Born, L. Besedovsky; 1University of Tübingen, Tübingen, Germany, 2University of Lübeck, Lübeck, Germany, 3University of Copenhagen, Copenhagen, Denmark.

An efficient T-cell effector immune response requires a strong adhesion of T cells to their targets, e.g., to virus-infected cells. This adhesion is dependent on the immediate activation of β2-integrins upon T-cell receptor (TCR) engagement by cognate peptide presented by major histocompatibility complex molecules (pMHC). GalpHA,-coupled receptor agonists are known to have immunosuppressive effects, but their impact on TCR-mediated integrin activation is unknown so far. We used soluble multimers of pMHC and ICAM-1, the ligand of β2-integrins, to assess the effect of different GalpHA,-coupled receptor agonists (including cathecolamines, prostaglandins (PGs), adenosine, histamine and serotonin) on TCR-induced integrin activation of human cytomoglobinoma cell line (Mφ)- and Epstein-Barr (EBV) virus-specific CDB T cells. We show that isoproterenol, epinephrine, norepinephrine, PGF2α, PGE2, PGD2, and adenosine strongly inhibit TCR-mediated integrin activation of antigen-specific CDB T cells in a dose-dependent manner, already at physiological concentrations. Using sleep as a natural condition of low levels of GalpHA,-coupled receptor agonists, we found that nocturnal sleep upregulates TCR-induced integrin activation compared to nocturnal wakefulness. The effect was slightly more pronounced for CMZC-specific T cells, for which it was the strongest. Importantly, for chemokine (C-C motif) CC receptor 7 (CCR7) it was found that it is increased in both early and late differentiated subsets. Our findings indicate that conditions characterized by low levels of GalpHA,-coupled receptor agonists, such as sleep, support the formation of immunological synapses and thereby enhance T-cell responses. The results might also be relevant to a variety of pathologies associated with increased levels of catecholamines (during chronic stress or sleep disturbances), PGs (tumor growth or malaria) and adenosine (hypoxia, sleep apnea, or tumor growth).

P.B3.03.05 LAT transmembrane domain in the plasma membrane of T cells

D. Glatzová1, T. Chum1, J. Kralová1, L. Cwikel1, T. Brdiková1, M. Cebecauerová1; 1Heyrovsky Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic, 2Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic.

LAT is transmembrane adaptor protein essential for T cell development and function. It is phosphorylated by ZAP70 protein tyrosine kinase upon T cell receptor activation and recruits multiple adaptor proteins to form a multisubunit signalling complex. It contains a very short extracellular region, transmembrane domain (TMD) and tyrosine-rich cytoplasmic tail. LAT is palmitoylated on two conserved cysteines (a-amino acids 26 and 29). Furthermore, TMD of LAT contains two proline amino acids (positions 8 and 17) function of which is still unknown. In agreement with the literature about other proteins, our MD simulations of LAT TMD demonstrate that the presence of prolines disrupts α-helical structure and causes so-called proline-kinks in its structure. Expression of mutants with prolines changed to alanines or in combination with mutants lacking the palmitoylation sites suggest their importance for sorting of LAT to the plasma membrane and their impact on T cell signalling.

Acknowledgement: We would like to acknowledge funding by GA UK project no. 288216. This work was also supported by Czech Science Foundation (15-06898S).

P.B3.03.06 Human double-negative regulatory T cells induce a metabolic switch in effector T cells by suppressing mTOR activity

T. Hauk, M. Augier, H. Bruns, A. Mackensen, S. Voikl; Dept. of Internal Medicine 5 – Hematology/Oncology, Erlangen, Germany.

The recently discovered subpopulation of TCRβ-CD4/CD8- (double-negative, DN) T cells are highly potent suppressor cells in mice and human. In murine transplantation models, adoptive transfer of DN T cells specifically inhibits alloreactive T cells and prevents development of transplant rejection or Graft-versus-Host-disease (GvHD). Interestingly, clinical studies in patients who underwent stem cell transplantation reveal an inverse correlation between the frequency of circulating DN T cells and the severity of GvHD, suggesting a protective role of DN T cells. Investigating the impact of DN T cells on effector T cells, we show that DN T cells diminish upregulation of glycolytic machinery, expression of glucose transporters and glucose uptake. In contrast, the uptake of fatty acids stays unchanged, indicating that DN T cells induce a metabolic switch in effector T cells. Consistent with this finding, DN T cells selectively inhibit the metabolic key regulator mTOR and enforced activation of mTOR in effector T cells with a chemical activator reversed the suppression of DN T cells. Hence both, mTOR activity and cell metabolism are crucial in DN T cells. Given that both, mTOR activity and cell metabolism are crucial in DN T cells, future studies are required to investigate the role of DN T cells in mediating suppression and deeper understanding of DN T-cells may have important implications for using them as a cellular-based therapy to limit alloimmune reaction.

P.B3.03.07 Rolling circle translation of a circular RNA in T helper lymphocytes

G. A. Heinz1, C. L. Tran2, P. Durek1, F. Heinrich1, Z. Fang1, C. Hoffmann1, V. Treffner1, W. Chen1, H. D. Chang1, A. Oastecek-Lederer1, D. H. Oastecek1, A. Radbruch1, M. F. Masheghi1; 1Deutsches Rheuma-Forschungszentrum, Berlin, Germany, 2Max Delbrück Center for Molecular Medicine, Berlin, Germany, 3Medical Faculty, RWTH Aachen University, Aachen, Germany.

Circular RNAs (circRNAs) have gained considerable interest in the course of their re-characterization as endogenous RNA species which is present in various cell types including T cells. However, the function and biological impact of circRNAs on cellular mechanisms still remain poorly understood. Analyzing total RNA sequencing data from naive and activated murine T helper lymphocytes under Th1, Th2 and Th17 polarizing condition, we discovered a highly abundant circRNA (ciri1167907). This circRNA candidate showed high expression in naive T helper cells, which strongly decreases with T cell activation and is further reduced upon repeated activation of Th1 cells. The corresponding mRNA expression follows a similar pattern, yet with a stable intermediate expression in Th1 cells. Interestingly, we identified one transcript variant in Th1 cells that harbors an alternative exon 1 and resembles the drastically reduced expression upon T cell activation. This suggests that the ciri1167907 might be generated from the alternative transcript rather than from the protein coding mRNA. Dissecting the ciri1167907 sequence, we found that it contains an infinite open reading frame with a potential for rolling circle translation. Taken together DN T cells impair metabolic reprogramming of effector T cells by abrogating mTOR signaling, thereby inducing a quiescent phenotype. These results uncover a new manner of DN T cell mediated-suppression and deeper understanding of DN T-cells may have important implications for using them as a cellular-based therapy to limit alloimmune reactive responses.

P.B3.03.08 Development of gene knock-down method in primary T cells

J. Hills, S. Kidger; CRUK-TDL, Cambridge, United Kingdom.

Genetic manipulation is an important tool in target validation. Through knock-down and overexpression of genes we can determine effects on biomarkers, determine synthetic lethabilities and investigate phenotypic effects. In the construction of mutants, constructs can illuminate the function of putative protein domains.

Genetic manipulation of primary immune cells is known to be challenging. These cells often have very specific culture conditions required for survival and proliferation, and can be quite sensitive to additional reagents. In addition to this, primary immune cells do not easily take up lipid particles or extracellular DNA. This characteristics make them particularly difficult to genetically manipulate by common methods such as transfection with lipid particles.

We have employed electroporation as a method of delivery of siRNA into primary T cells. Using this method, we can achieve consistent knock-down of at least 70% at both mRNA and protein levels, whilst maintaining good viability. So far, this method has been optimised for use in activated CD3+ CD8+ and expanded Treg populations. Ongoing work will expand this knock-down method to further T cell subsets, including unstimulated cells, to allow for wider target validation work. We also intend to develop a robust method of overexpression in primary T cells, which would allow us to investigate protein domain function.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Circular RNAs are abundantly expressed in a subset-specific manner in T helper lymphocytes


1Deutsches Rheuma-Forschungszentrum, Berlin, Germany, 2Max-Delbrück-Center for Molecular Medicine, Berlin, Germany.

Circular RNA represent a class of covalently closed RNA molecules, which are spliced from primary transcripts in a head-to-tail fashion and generally considered to be non-coding. Due to their ring structure, they lack both 5’ cap and poly-A tail and display exonuclease resistance. Next-Generation Sequencing (NGS) data revealed that circRNAs are abundant and display development- and stage-specific expression patterns, although little is known about the function of most identified circRNAs.

Here, we hypothesize that circRNAs play a role in the function of proinflammatory T helper (Th) cells, which are involved in the initiation and maintenance of chronic inflammatory diseases.

Using NGS, we assessed the global expression profile of circRNAs in naive, once and repeatedly activated Th cells, mimicking protective and proinflammatory Th cells, respectively. As a result, 41,169 potential circRNAs were detected in Th cells. Of the highly expressed circRNAs (>50 reads), the majority consists of one to three exons with an overall expression level of 60% or more, with a 80% bound to their start sites and is largely expressed in all Th subsets with the highest number in naive Th cells. However, their expression is subset- and activation-dependent with overall higher expression in naive Th cells. We selected several circRNAs which were highly expressed and differentially regulated in Th cells. Using Northern Blot, RT-PCR and RNA FISH we validated these candidates, verified their RNAseq expression and confirmed their location in the cytoplasm. Our data suggest that circRNAs are abundantly expressed and might have a regulatory function in proinflammatory Th lymphocytes.

Ig-like transcript 2 (IT2) suppresses T cell function in Chronic Lymphocytic Leukemia

M. Villa-Álvarez, S. Loreto-Herrera1,2, A. González-Rodríguez1,3, A. Lopes Sato1,4, A. Payet, S. E. González-García, L. Huerto-Zapico, S. González1,2,4,5;

1University of Oviedo, Oviedo, Spain, 2Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Oviedo, Spain, 3Instituto de Investigación Bioassituatiorial del Principado de Asturias (IISPA), Oviedo, Spain, 4Hospital Universitario Central de Asturias, Oviedo, Spain, 5Hospital de Cabueñes, Gijón, Spain.

Introduction Chronic lymphocytic leukemia (CLL) is associated with a profound dysregulation of the immune system. Loss of T cell function is frequently caused in cancer by sustained signaling of inhibitory receptors. In this work, we analyzed the role of the inhibitory receptor Ig-like transcript 2 (IT2) in the pathogenesis of CLL. Methods The expression and function of IT2 and its ligands in different lymphocyte subsets from 52 CLL patients and 20 healthy donors were evaluated in this study. Results IT2 expression was markedly reduced on leukemic cells, whereas it was increased on T cells from CLL patients, particularly in patients harboring chromosome 11q deletion, which includes the IT2 gene. The expression of IT2 ligands in leukemic cells was also deeply dysregulated. IT2 impaired the activation and proliferation of CD4 and CD8 T cells in CLL patients, but it had no effect in leukemic cells. ITL2 downregulated the production of IL-2 by CD4 T cells of CLL patients and induced the expression of cytokines that promote the survival of leukemic cells, such as IL-10 and IL-6, in an IT2-dependent manner. In addition, ITL2 blockade restored the activation, proliferation and cytokine production of T cells. Conclusion Our experiments indicate that ITL2 impairs T cell function in CLL. Here, we describe a novel immune inhibitory pathway that is up-regulated in CLL and delineate a new potential target to be explored in this disease. Funding: This work was supported by the Spanish grants of Fondo de Investigaciones Sanitarias (Instituto de Salud Carlos III) PI12/01280 and PI16/01485.

The effects of redox regulated cofilin-1 on T-cell mediated immunity

X. Yang, I. Seeland, B. Jóhanns, G. Kübbéck, G. Wabnitz, J. Kübbéck, Y. Samstag;

1Institute of Immunology, Heidelberg, Germany, 2German Cancer Research Center, Heidelberg, Germany.

Cofilin-1 is an actin-remodeling protein, which is essential for T cell development, activation and migration. Oxidation of cofilin-1 causes it to lose its affinity for actin and to translocate to the mitochondria, which leads to T cell hypo-responsiveness or even programmed cell death. This includes both oxidation of cofilin-1 at Cys residues and dephosphorylation at Ser 3.

In terms of this, we hypothesized that cofilin-1 Cys to Ala mutants may make T cells more resistant to oxidative stress. Therefore, we investigated the functionality and regulatability of different non-oxidizable Cys to Ala mutants expressed in human peripheral blood T cells under oxidative stress conditions. We identified a double Cys to Ala mutant that showed notable resistance towards cytotoxic H2O2 effects. Still, this mutant differentially rescued T cells under oxidative stress conditions with regard to sustained adhesion of T cells to APCs and formation of the immunological synapse, the phosphorylation and actin-remodeling functions of cofilin-1, T cell activation and migration.

Furthermore, we have generated T cell specific knock-in mice which express these cysteine-to-alanine mutants instead of the wild type cofilin-1. These mice will serve as tools to clarify the role of cofilin-1 redox-regulation during T cell responses in vivo and its potential role in different immune-related diseases (e.g. infections, tumor immunology, or chronic inflammation).

T-cell regulation - Part 4

Superior contact avidity of Treg membrane orchestrates antigen specific suppression via stripping cognate peptide-MHCII from DC surface


NIH, Bethesda, United States.

Tregs are professional suppressors of the immune response, yet their mechanism of action in vivo remains unclear. We compared stoichiometry of the interactions of activated SCCT T cells and Th17 T cells with MCHCII pulsed splenic DCs by electron and confocal microscopy. Image analyses revealed that Tregs displayed a distinct morphology with finger like membrane projections at the DC binding site and uropods at the rear end within three hours of co-culture. In contrast, activated T cells maintained their round morphology. Tregs occupied a greater extent of the DC surface than activated T cells consistent with a higher binding avidity. Intravital two-photon microscopy of adoptively transferred OTII Tregs and activated OTII T cells demonstrated that Tregs displayed greater volume and duration of contact with OVA323-339 pulsed DC compared to that of activated cells. Subsequent to their high avidity interactions, SCCT Tregs captured MCHCII-I-E complexes from DC surface reducing the amount of MCC-p presented on DC. Reduced antigen presentation was not due to global suppression of the DC's ability to present antigen as SCCT and 3A9 Tregs only captured their cognate peptide-MHCII from MCCHEL double pulsed DC, reducing the DC presentation in an antigen specific manner. When double pulsed DCs were cultured with Treg specific for one peptide, separated from the Tregs, the DC failed to prime T cells specific for the antigen seen by the Treg, but primed T cells specific for the second antigen. Altogether, we propose antigen specific depletion of peptide-MHCII complexes as a new mechanism for Treg mediated suppression.

Immunophenotyping of suppressor and cytotoxic lymphocyte subsets and cytototoxic mechanisms in non-small cell lung cancer


1İstanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, İstanbul, Turkey, 2Istanbul University, Cerrahpaşa Medical School, Department of Thoracic Surgery, İstanbul, Turkey, 3Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, İstanbul, Turkey, 4Istanbul University, Cerrahpaşa Medical School, Department of Medical Oncology, İstanbul, Turkey, 5Istanbul University, Cerrahpaşa Medical School, Department of Internal Medicine, İstanbul, Turkey.

Lung cancer is the leading cause of cancer-related death worldwide and non-small cell lung cancer (NSCLC) is the most common form of it. The aim of this study was to assess immunological properties of circulating T lymphocyte subsets and natural killer (NK) cells. Thirty newly diagnosed patients with T1-3N0M0 NSCLC, with a history of preoperative chemotherapy and/or radiotherapy, and 46 healthy subjects were included. Lymphocyte subsets were analyzed by flow cytometry. Cytotoxic capacity of NK and CD8+ T cells were evaluated by CD107a degranulation assay. Compared to healthy subjects, NK, NKT, CD3+HLADR+ and CD8+CD28 suppressor T cells were significantly increased, while percentage of CD8+CD28+, CD4+ and CD3+ T lymphocytes were significantly decreased in patients with NSCLC (p<0.002, p<0.005, p<0.000, p<0.001, p<0.001, p<0.02 and p<0.02, respectively). Although the cytotoxic capacity of NK cells were similar between the groups, increased CD107a expression was observed in CD8+ T cells in stimulated and unstimulated groups (p=0.001). The rates of lymphocyte and NK subsets were not significantly different between earliest stage (T1) patients and patients with T2-3 (p>0.05).Increased CD8+CD28+ T cells might suppress antitumor immunity. Although the number of NK cells were increased in NSCLC group, their cytotoxic capacity was impaired. An immunological scoring system might contribute to better understanding of the prognosis of NSCLC. The role of immune cells in different stages of cancer needs to be further studied.
Cellular metabolism is a critical factor in immune cell activity. It has been well studied in conventional T cells (T_{con}), where mTORC1 promotes anabolism during antigenic stimulation while AMPK favors catabolism to support memory function. Regulatory T cells (T_{reg}) are essential to maintain immune tolerance. Although they have been extensively studied, little is known about their metabolic profile. We therefore developed a model where AMPK was selectively deleted in T_{reg} (Foxp3^{+/+} Prkaa1-/- mice). These mice were hyperglycemic and had increased weight. While T_{con} showed increased mitochondrial and palmitate harnessing as well as increased mTORC1 activity. In a tumor context, the proportion of tumor-infiltrating T_{reg} was decreased in Foxp3^{+/+} Prkaa1-/- mice which correlated with a better activation of T_{reg} and slowed tumor growth. Analysis are underway to further evaluate these metabolic changes and their impact on the stability, migration and survival of tumor-infiltrating T_{reg}.

P.B3.04.03

**AMPK pathway in regulatory T cell is critical to maintain oxidative metabolism and keep energy balance within the tumor micro environment**

J. Djouven, R. Vailion, M. Laviron, E. Ronin, S. Gregoire, B. Salomon;

Regulatory T-cells (Treg) are a subpopulation of CD4+ T-cells, associated with immunosuppression and preservation of self-tolerance. Treg can suppress activation of effector T-cells through various mechanisms, including the degradation of ATP into the suppressive moleculeadenosine through the enzymes CD200 and CD73. The mechanisms regulating CD200 and CD73 on CD4+ T-cells are yet unknown and is independent of FOXP3. Previous studies in tumor cells have described that inhibition of autophagy leads to the up-regulation of CD200 expression. Thus, we hypothesized a similar correlation in T-cells. Consequently, we investigated signals leading to the de novo induction of CD200 on Treg and assessed the influence of chloroquine, an autophagy inhibitor, on this process. Naive CD4+CD25+CD90+ T-cells were activated in vitro with anti-CD3/CD28 coated microbeads in the presence of IL-2 and TGF-β to trans retinoic acid (atRA) with or without chloroquine. First experiments revealed that TGF-β/atRA led to a stronger induction of CD200 on the CD25+/CD73+ Treg population compared to culture in IL-2 alone. The combination of TGF-β/atRA+ chloroquine boosted CD200 and FOXP3 expression, and led to a stronger suppressive function in vitro compared to TGF-β/atRA Treg induction without chloroquine. Intriguingly, T-cells cultured in chloroquine without TGF-β/atRA did not induce Treg. Thus, chloroquine synergizes with TGF-β/atRA signaling to enhance tolerogenic functions during Treg induction. The exact molecular mechanisms underlying these observations will be the focus of further studies. In conclusion, our studies reveal that additional signals may influence induction of Treg by TGF-β/atRA which may allow to develop strategies for the manipulation of T-cell tolerance.

P.B3.04.05

**Role of the zinc-finger protein MAZR during NK1 T cell lineage differentiation**

1Institute of Pathophysiology, Infectology and Immunology, Medical University Vienna, Vienna, Austria; 2Institute of Specific Pathology and Tropical Medicine, Medical University Vienna, Vienna, Austria.

Metalloregulatory zinc finger protein MAZR, also known as PATZ1, is a transcription factor that is expressed at high levels in NKT1 cells, a small subset of CD8+ T cells. MAZR overexpression in the pre-T cell line Jurkat led to enhanced production of granzyme B, an enzyme involved in cell-mediated cytotoxicity. MAZR deficiency in NKT1 cells was associated with reduced granzyme B expression, suggesting a role for MAZR in the development and function of NKT1 cells. To further investigate the role of MAZR in NKT1 cell development, we generated a transgenic mouse line expressing a fluorescent protein (eGFP) and Cre recombinase under the control of the Hobit promoter. Hobit is a transcription factor that is highly expressed in NKT1 cells and plays an essential role in the development of the NKT1 lineage, but not in other NKT lineages. We did not find YFP expression in NKT1 and NKT2 cells, suggesting that Hobit+ NKT1 cells lack NKT2 and NKT17 potential. Thus, our data show that NKT1 forms an independent subset of terminally differentiated NKT cells in the peripheral organ.

P.B3.04.04

**Chloroquine promotes up-regulation of CD39 expression and suppressive function during TGF-beta mediated Treg induction**

M. C. Germer, L. Ziegler, R. L. Schmidt, K. G. Schmutterer;
Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria.

To address the differentiation pathway of NKT1 cells, NKT2 cells and NKT17 cells expressed Hobit. To examine the potential of thymic precursors to generate NKT1 cells, we depleted tdTomato+ NKT1 cells using DT and when this was done, mature NKT1 cells in contrast to immature NKT1 cells, expressed Hobit. To analyse the re-appearance of these cells, we found that mature NKT1 cells did not re-develop after depletion, suggesting that the population is stably maintained in the periphery. We analyzed the re-appearance of these cells. We found that mature NKT1 cells did not re-develop after depletion, suggesting that the population is stably maintained in the periphery. To address the differentiation pathway of NKT1 cells, NKT2 cells and NKT17 cells expressed Hobit. Thus, our data show that NKT1 form an independent subset of terminally differentiated NKT cells in the peripheral organ.
Human CD8+HLA-DR+ regulatory T cells share phenotypic and functional features with classical CD4+Foxp3+ regulatory T cells

A. Machicote, S. Belén, P. Baz, L. A. Bilardo, L. Fainboim,
Laboratorio de Inmunogenética, Instituto de Inmunología, Genética y Metabolismo, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-Campo Nacional de Investigaciones Científicas y Técnicas, CABA, Argentina.

Introduction: We previously described a human CD8+ regulatory T cell subset, constitutively expressing the HLA-DR molecule (CD8+HLA-DR+ Tregs), which suppresses the proliferation of autologous PBMCs through cell contact and CTLA-4. We aim to characterize the regulatory signature of these Tregs, to identify additional mediators involved in their regulatory mechanism, and to evaluate their exhaustion status.

Materials and Methods: Peripheral and cord blood samples were obtained from healthy donors. Phenotypic markers were analyzed by flow cytometry. Suppression assays were performed with CellTrace Violet-stained PBMCs as responders, co-cultured with sorted CD8+HLA-DR+ or CD8+HLA-DR- cells, and activated with aCD3/αCD28. Cytokine secretion was analyzed after PMA/ionomycin stimulation.

Results: In comparison with CD8+HLA-DR- cells, CD8+HLA-DR+ Tregs showed an increased frequency of PD-1 (p=0.0001) and TIGIT (p=0.0001), and lower expression of CD127 (p=0.0009). Consistent with the high expression of PD-1, the addition of PD-1 and PD-L1 neutralizing antibodies abrogated the suppression effect of CD8+HLA-DR+ Tregs (p=0.0015), acting preferentially on CD8+ responder cells. In comparison with CD8+HLA-DR- cells, CD8+HLA-DR+ Tregs presented a higher frequency of IFNγ (p=0.0098), TNFa (p=0.0004) and CD107a (p=0.0006) after PBMCs stimulation, and a higher proliferation rate (p=0.0037). Within adult PBMCs, 90±2% of CD8+HLA-DR+ Tregs have a differentiated- or memory-like phenotype, characterized by higher IFNγ and TNFa production.

Conclusions: CD8+HLA-DR+ Tregs show high similarities with classical CD4+Foxp3+ Tregs, including expression of PD-1, TIGIT and CD127 molecules, and abrogation of suppressor capacity by anti-PD-1 antibody. Our results also confirmed that CD8+HLA-DR+ Tregs are not exhausted cells.

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POSTER PRESENTATIONS

P.B3.04.14
The role of anergy-related molecules in development and function of regulatory T cells induced by B cells
T. Yeh, B. Chiang;
Graduate Institute of Immunology, Taipei, Taiwan.

Introduction: Naive B cell could act as antigen presenting cell to convert CD4+CD25+ T cell into FOXP3+ regulatory T cell which called Treg-of-B cells. Treg-of-B cell suppressed effector T cell proliferation by cell-cell contact manner which is different from other Treg population, included Treg, T1R and Th3. In several studies of autoimmune disease animal models, Treg-of-B cell could alleviated inflammation. Anergy is a mechanism of peripheral tolerance which caused by weak or non-costimulatory signal. Treg are considered to be naturally anergic because of its hypoproliferative characteristics and low IL-2 production with involvement of anergy associated factors. Treg-of-B cells have been found also with the anergic phenotype. Materials and Methods: In this study, we aim to identify the anergic phenotype of Treg-of-B cells, and analyze the anergy associated genes expression. We established several induction systems to discuss how antigen specificity and costimulatory signal affect Treg-of-B cell development and function. Then we will further analyze the IL-2 suppressor - Aiolos expression of Treg-of-B cell. Results: Treg-of-B cell exerted anergic phenotype but did not express NFAT associated anergic factors. Treg-of-B cell induced with antigen specific B cell or higher costimulatory signal slightly restored the suppressive function and accompanied with decreased Aiolos expression. Conclusions: Antigen specificity and high costimulatory signal caused Treg-of-B cell to be more activated which affect their suppressive function. Different expression of Aiolos suggested that it might be associated to the anergic status of Treg-of-B cell. but whether Aiolos involved in development and function of Treg-of-B cell still needs clarification.

P.B3.04.15
Characterization of the FOXP3-regulated anti-proliferative gene signature in thymus-derived regulatory T-cells
L. Ziegler, M. Gerner, R. Schmidt, K. Schmetterer;
Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria.

Background: The transcription factor Forkhead-box protein 3 (FOXP3) is master regulatory factor for thymic-derived regulatory T-cells (Treg) and controls the expression of several hundred genes. Still our knowledge about this FOXP3-regulated gene signature is far from complete. Using microarray screenings of FOXP3 transgenic human T-cells, we identified several genes, which are regulated by FOXP3 and have been described as cell-cycle regulators in various cell types: Juxtaposed with another zinc finger 1 (JAZF1), MAX-interacting protein 1 (MX1), B-cell translocation gene 1 (BTK1) and Glucocorticoid-induced leucine zipper (GLIZ). Methods: Expression of these genes was measured by RT-PCR in resting or anti-CD3/anti-CD28 activated FOXP3+ or control-vector transduced T-cells or CD4+CD25+ Treg. cDNAs for the genes were cloned into a retroviral expression vector and the effects of overexpression in CD4+ T-cells on proliferation, marker expression and cytokine secretion were measured. Results: Expression of all four genes was induced in FOXP3-transgenic T-cells and Treg following activation. In contrast, expression in control-vector transduced or CD4+ effector T-cells was significantly down-regulated. Overexpression of GLIZ showed a marked up-regulation of the Treg markers CD25 and GITR. Following activation, proliferation of all transgenic T-cells was significantly decreased. All four genes showed effects on the expression of several different cytokines, including IL-1, IL-17 and IFN-γ. Conclusion: JAZF1, MX1, BTK1 and GLIZ are involved in the maintenance of hypo-responsiveness in Treg and contribute to their reduced cytokine secretion. Thus, our data add to the understanding of the FOXP3-regulated gene signature of these cells and Treg and may open new strategies for the manipulation of these cells.

P.B3.04.16
Platelets induce a regulatory phenotype in T cells via the expression of GARP
N. Zimmer, S. A. Hahn1, S. Wilden1, E. Walter1, K. Jurk1, S. Grabbe1, A. Tuettenberg1;
1Department of Dermatology, Mainz, Germany; 2Center for Thrombosis and Hemostasis Mainz (CITH), Mainz, Germany.

Introduction: Beside their main function in hemostasis, platelets are important modulators of the innate and adaptive immunity through their interaction with immune cells. GARP, Glycoprotein A, Retractin, Protein, is a member of the IL-10R family, and a modulator for the development of T regulatory cells (Treg). It is known to be involved in the induction of TGF-beta and is therefore involved in the regulation of the peripheral immune responses. Recently, we were able to show that the soluble form of GARP (sGARP) has strong regulatory and anti-inflammatory properties in vitro and in vivo. sGARP leads to induction of peripheral Treg (pTreg) as well as to inhibition of tumor antigen-specific CD8+ T-cells. Materials and Methods: In the present study, we investigated the effect of platelets on the differentiation and phenotype of CD4 T cells dependent on GARP. Results: sGARP was detected in the supernatant of activated platelets. Consequently, platelets were able to inhibit dose dependently the proliferation and cytokine production, while inducing a strong Foxp3 expression and a suppressive capacity in coculture. Using a blocking anti-GARP mAb, we were able to reverse these effects.

Conclusion: Our data give evidence that platelets are capable to induce pTreg in a GARP-dependent manner. The expression and shedding of GARP by platelets could be of importance in diseases like cancer, where poor prognosis and metastasis are associated with elevated numbers of circulating platelets (thrombocytosis).

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P.B3.04.17
Tr1-like (IL-10+, IFNg-, IL4-) cells in peripheral blood are part of the T effector memory pool and are preferentially stimulated via CD55
I. A. Charles, J. M. Ramage, I. Spendlove, Cancer Immunotherapy group;
The University of Nottingham, Nottingham, United Kingdom.

Effector T cells arise from naïve T cell stimulation and enter a long term memory pool. While they can adapt their function to specific environmental cues, sometimes called plasticity, their phenotype remains broadly fixed. Unlike natural T regulatory cells that emerge from the thymus with a defined phenotype (CD4+CD25+), Tr1-like T cells, are induced on the basis of the GARP molecule, and have a hypo-proliferative and anti-inflammatory phenotype. In this study, we attempted to identify markers associated with the hypo-proliferative and anti-inflammatory phenotype of these cells. We demonstrate that the IL-10 producing T cell, Tr1-like T cells, are induced on the basis of the GARP molecule, and have a hypo-proliferative and anti-inflammatory phenotype.
POSTER PRESENTATIONS

P.B4.01 T-cell activation and exhaustion - Part 1

M. Surce1, C. Constantin2, R. Huica1, A. Munteanu1, I. Pirvu1, G. Iovaru1, O. Bratu1, D. Ciota1, C. Ursuc1, M. Neagu1,2
1Victor Babes National Institute of Pathology, Bucharest, Romania; 2Coimbra University Hospital, Bucharest, Romania.

Cutaneous melanoma (CM) represents only 4% of all cancers, but it is responsible for 80% of skin cancer deaths, thus identifying new prognostic and evolution markers remains a constant endeavor. Methods. Using flow-cytometry peripheral blood mononuclear cells (PBMCs) were analyzed by antigen-specific and non-specific IFN-γ and TNF-α production. Results. Phenotypic analysis revealed that T cells from CM patients exhibited a higher percentage of CD8+ T cells compared to normal skin. Conclusions. The results suggest a potential role of T-cell activation in CM, which could be further explored for diagnostic and therapeutic purposes.

P.B4.02 Effective expansion and reprogramming of tumor infiltrating lymphocytes from non-small cell lung cancers

R. De Groot1, M. M. van Loenen1, A. Guislain1, M. M. van den Heuvel1, J. de Jong1, R. M. Spaapen1, D. Amsen2, J. H. Haanen1, K. Monkhorst3, K. J. Hartemink4, M. C. Walkers1
1Sanquin Research, Amsterdam, Netherlands; 2Netherlands Cancer Institute, Amsterdam, Netherlands.

Non-small cell lung cancer (NSCLC), the second most occurring type of cancer, is highly recurrent with limited 5-year survival. NSCLCs contain high numbers of T cells, and although suggestive of tumor reactivity, these tumor infiltrating lymphocytes (TILs) are unable to eradicate the cancer. Here we investigated whether TILs from NSCLC can be reprogrammed and autologously used for cell therapy. TILs were isolated from tumor tissues and reprogrammed via in vitro activation using a standard TIL expansion protocol. Strikingly, the vast majority of expanded TILs from primary NSCLC tumor tissues (stage I-IIIA) were tumor reactive; TILs from 13/17 donors (76,5%) produced inflammatory cytokines in response to tumor digests, but not in response to distal non-tumorous lung tissues. Tumor responses ranged from 1%-35% of cytokine-producing TILs, which correlated with expression of activation markers CD137 on tumor-reactive T cells and CD40L on tumor-reactive CD4+ T cells. Importantly, TIL cultures with high cytokine responses contained polyfunctional T cells that produced IFN-γ together with TNF-α and/or IL-2. Strikingly, high expression of CD103+CD69+ on CD8+ T cells, and the presence of B cell as well as regulatory T cell infiltrates in the tumor were strong predictors of the intensity of tumor reactivity in expanded TILs. Our data show that reprogramming of TILs from NSCLC is effective, strongly suggesting that autologous T cell therapy should be considered to treat NSCLC patients.

P.B4.03 The SPPL3-controlled tumor glycosphingolipid repertoire determines MHC class I functionality

A. A. de Waard1, M. J. Longma1,2, M. Radben1, T. Zhang1, V. A. Blomen1, R. Platzer1, S. Blais2, H. Holst1, R. Schatzmaier2, L. J. Iansens3, A. Mulder1, M. H. Heemskerk3, F. H. Clos4, M. Griffioen1, H. S. Overkleeft1, J. B. Huppler1, M. Wouda1, T. Brummelkamp2, I. Neefjes2, R. M. Spaapen1,1
1Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands; 2Leiden University Medical Center, Leiden, Netherlands; 3Netherlands Cancer Institute, Amsterdam, Netherlands; 4Institute for Hygiene and Applied Immunology, Vienna, Austria.

We identify the intramembrane protease SPPL3 as a novel positive regulator of tumor glycosphingolipid (GSL) repertoire, controlling tumor cell MHC-I functionality. Additional genome-wide screens revealed that SPPL3 controls enzymes of the GSL synthesis. Indeed, mass spectrometry of the GSLs showed that the repertoire of SPPL3-null tumor cells is different from the repertoire of wild-type tumor cells. In agreement, we find that SPPL3 is required for the proteolytic inactivation of B3GNT5, an enzyme involved in the synthesis of lactoseries GSLs, leading to a decrease or absence of lactoseries GSLs. Moreover, we show that SPPL3 controls two enzymes that control the synthesis of β3GNT6-derived GSLs. In conclusion, SPPL3 controls the tumor glycosphingolipid repertoire, which determines MHC class I functionality, and this can be exploited therapeutically to enhance tumor immunogenicity.

P.B4.04 Tumor infiltrating T cells: complete workflows allow faster and improved flow cytometric analysis of syngeneic mouse tumors

C. Evaresto1, R. Siemer, A. Agoiku1, J. Brauner, O. Hardt, C. Dose, A. Richter,
1Miltenyi Biotec, Bergisch Gladbach, Germany.

Immune therapies have proven clinical efficacy in multiple cancers. Syngeneic mouse models are highly predictive and are used to evaluate the effects of new immune therapies. However, the analysis of these models is often time-consuming and labor-intensive. Here, we present complete workflows combining tissue-storage, dissociation, T cell isolation and phenotyping. Tissues were processed immediately after sacrifice or stored in formalin at 4°C. Tumor-dissociation was automated and optimized for epiteope preservation using a tissue dissociator (gentleMACS® Octo). Phenotypic analysis revealed that optimal metabolic screening was essential for analysis of critical tumor-specific sub-populations, such as CD11b+CD11c+ T cells. We developed new reagents which enrich for rare tumor infiltrating T cells up to 500-fold, while maintaining activation status and phenotype. Importantly, use of recombinant REAfinity™ antibodies significantly diminished non-specific labeling of cells present in the tumor microenvironment. Finally, flow cytometric analysis was performed using an automated analyzer (MACSQuant X™). This instrument decreased total and hands-on acquisition time by facilitating fast and automated sample processing, including sample mixing and cell counting. In conclusion, we optimized workflows including standardized processing of tumor samples, new tools for TIL isolation and automated flow cytometry. These workflows reduce experimental time and allow the performance of more complex experiments. Use of these tools can significantly increase the data quality in immuno-oncology and immunotherapy research.

P.B4.05 Immune profiling of the tumour microenvironment in cancer to determine patient responses to immunotherapy

A. L. Ferguson1, J. Tohill1, K. Lo2, K. A. Hong3,4, G. Long5, R. Scally6,7, U. Palendira8
1Centenary Institute, Sydney, NSW, Australia; 2Sydney Medical School, The University of Sydney, Sydney, NSW, Australia; 3Westmead Hospital, Sydney, NSW, Australia; 4Department of Anatomical and Cellular Pathology at the Chinese University of Hong Kong, Hong Kong, Hong Kong; 5Royal Prince Alfred Hospital, Sydney, NSW, Australia; 6Melanoma Institute Australia, The University of Sydney, North Sydney, Australia.

Cancer immunotherapy targeting immune checkpoints and inhibitory molecules is a promising treatment option for many types of cancer. However, durable response are seen in less than 10% of patients and many experience immune-related adverse events such as the development auto-immune disease. Recent advances in tumo immunotherapy research have found that certain immune cell subsets such as CD103+ tumour-resident CD8+ T cells are associated with improved survival in some cancers. It has become clear that the more we understand about the immune profile of the tumour micro-environment (TME) the more we will be able to predict a patients response and therefore direct the choice of immune-therapy in the clinic. In order to profile the resident immune cells and presence of immunotherapy targets in the human TME, we employed a technique that could efficiently utilise both high-dimensional mass cytometry and spatial analysis, imaging mass cytometry (IMC). Using formalin fixed paraffin embedded human tumour tissues we designed an IMC panel to enable high-throughput immune profiling of prospectively collected tumours. This study provides novel information about the immune profile of the TME in multiple cancers. These findings may be crucial in determining better strategies of treatment for cancer in immunotherapy.
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P.B4.01.06

Intratumoral T cell exhaustion in a murine pancreatic cancer model: influence of induced regulatory T cells

A. Hanlon1, V. Lutz1, E. Landmann1, M. Huber1, M. Buchholz1, T. Gress1, C. Bauer2,1
1Division of Gastroenterology, Endocrinology, Infectology and Metabolism, University Hospital Giessen and Marburg, Philippus University Marburg, Marburg, Germany;
2Institute for Medical Microbiology and Hygiene, Philippus University Marburg, Marburg, Germany.

Introduction: Pancreatic cancer is one of the most aggressive malignancies with poor prognosis where new therapeutic approaches are needed. T cell exhaustion plays a critical role in at least some subtypes of pancreatic carcinoma. This CD8+ T cell (CTL) dysfunction has evolved as a paradigm to understand tumor escape from immune surveillance. We present a model system of adoptive T cell transfer in order to elucidate mechanisms of T cellular exhaustion and of ways to disinhume intratumoral T cells.

Methods: We work with the OVA model antigen system. Antigen-specific CTL were generated from transgenic OT-I mice and transferred into tumor-bearing mice to investigate the role of induced regulatory T cells (Treg) on our CTL, antigen-specific Treg were co-transferred. Beside therapeutic response, ex vivo parameters were characterized, such as expression of co-inhibitory receptors (PDL-1, TIM-3, LAG-3, CTLA-4), Lamp-1-associated degranulation and intracellular production of IFN-γ and TNF.

Results: We have established growth characteristics of PancOva and intratumoral infiltration kinetics of effector CTL. Additionally, in vitro and in vivo characterization of iTreg behavior was performed and also their effect on cytotoxic T cell responses investigated. The induction of T cell dysfunction was exposed as an active, antigen- and contact-dependent process.

Conclusions: We intend to validate our results in an orthotopic mouse model to approximate to real cancer conditions. Furthermore, mechanisms of iTreg-induced T cell dysfunction will be investigated using FDUPI-IRF-γR (FIR) OT-I mice in an in vivo/in situ approach using a dorsal skinfold chamber model to visualize intratumoral T cells.

P.B4.01.07

CXCR5+PD-1+ CD8 T-cells in human blood and lymph nodes

T. Hofmann1, F. E. Remmerswaal1, F. Eremens1, M. Levin1, A. P. Kater1, S. H. Tonn1,2
1AMC Amsterdam, Amsterdam, Netherlands; 2Albert Schweitzer Hospital, Dordrecht, Netherlands, LUMMCARE, Amsterdam, Netherlands.

Re-investigating exhausted T-cells with immune checkpoint blockade (ICB) has shown promising efficacy in the treatment of several malignancies. However, response rates vary between patients, and it’s unclear which parameters predict clinical response to ICB. Recently, a subset of CXCR5+PD-1+ CD8 T-cells has been identified in mice. This memory-like population appears to be crucial for the expansion of CD8+ T-cells during ICB, and is characterized by the expression of transcription factor Tcf1. Although CXCR5+PD-1+ T-cells are detectable in both murine and human tumors, it’s unknown whether these cells have a similar phenotype and function in humans, it’s unknown whether these cells have a similar phenotype and function in humans.

To investigate CXCR5+PD-1+ CD8 T-cells, we analyzed human peripheral blood and lymph node samples from healthy donors with flow cytometry. We found CXCR5+PD-1+ CD8 T-cells to be more frequent in lymph nodes. CXCR5+PD-1+ CD8 T-cells resemble antigen-experienced memory-like cells, with high expression of KLRL1, CD127, granzyme B, and Eomes.

We show expression of effector molecules like granzyme B and T-bet. They also express high levels of PDL1, which is in accordance with the phenotype described in mice studies. We found CXCR5+PD-1+ CD8 T-cells within the EBV-specific T-cell population, but not within the FLU-specific T-cell compartment, showing that chronic antigens are required for this population to develop. To study their functionality, we are currently analyzing the transcriptional and functional capabilities of these cells in relation to ICB. These results can further elucidate the role for CXCR5+PD-1+ CD8 T-cells in clinical responses during ICB.

P.B4.01.08

TCR-activated T cells release cytokines that induce MDSC to express arginase-1, which does not suppress T cell proliferation

K. S. Kider;
Georgia State University, Atlanta, United States.

Although previous reports suggest that tumor-induced myeloid-derived suppressor cells (MDSC) inhibit T cell proliferation by Larginine depletion through arginase-1 activity, we herein show that arginase-1 is neither inherently expressed in MDSC nor required for MDSC-mediated inhibition of T cell proliferation. Employing Percoll density gradient centrifugation, large expansions of MDSC in the bone marrow of tumor-bearing mice were isolated and selected potent inhibition on T cell proliferation induced via TCR-ligation, Concanavalin A, PMA plus ionomycin, or IL-2. These MDSC, though suppressive toward T cell proliferation, do not hinder TCR-induced IFNγ secretion or granzyme B expression. Despite effectively inhibiting T cell proliferation, both G- and M-MDSC exhibit no arginase-1 expression; however, arginase-1 can be induced by exposure to TCR-activated T cells or their culture medium, but not by T cells activated by other means or growing tumor cells. Western blot analysis revealed that TCR-activated T cells confer arginase-1 expression in MDSC by secreting cytokines that manifest as two distinct signaling-relay axes, IL-6-to-IL-4 or GM-CSF/IL-4-to-IL-10. Specifically, IL-6 signaling increases IL-4R on MDSC, enabling IL-4 to induce arginase-1 expression; similarly, GM-CSF in concert with IL-4 induces IL-10R, allowing IL-10-mediated induction. Surprisingly, our data demonstrate that the induction of arginase-1 is not conducive, per se, for MDSC-mediated inhibition of T cell proliferation, as neither supplementation of L-arginine nor arginase-1 inhibitor rescue T cell proliferation. Furthermore, this inhibition relies upon direct cell contact that is not dampened by PD-L1 blockade or SIRPa deficiency.

P.B4.01.09

CTLA-4 (CD152) impairs cytotoxic T lymphocyte responses via PDCD4 induction

H. Lingel1, J. Wissing1, A. Arrar1, F. Klawonn1, M. Pierau2, L. Jänsch2, M. C. Brunner-Weinzierl1
1Otto-von-Guericke University, Magdeburg, Germany, 2Helmholtz Centre for Infection Research, Braunschweig, Germany.

Inhibitory T-cell surface receptors like Cytotoxic T lymphocyte-associated Protein-4 (CTLA4) and Programmed cell death 1 (PD-1) play a crucial role in the termination of adaptive immune response. CTLA4 functionally antagonizes the activity of CD8 T-cells and is being used in immune checkpoint therapy as a promoting approach to restore effective T-cell responses against tumors. However, the intracellular pathways triggered by these receptors still remain incompletely understood. To determine target molecules downstream of CTLA-4, an accurate mass spectrometry analysis was performed. The dataset revealed that the engagement of CTLA-4 led to altered phosphorylation of proteins involved in T-cell signaling, DNA replication, RNA processing and microtubule polymerization. Beside other targets, a CTLA-4-induced expression of the translational inhibitor Programmed cell death 4 (PDCD4) could be revealed and characterized. This mechanism was responsible for the restriction of cytotoxic T lymphocyte effector functions. Accordingly, the deficiency of PDCD4 led to superior control of melanoma growth in vivo. Furthermore, identified upstream elements suggest this pathway as a part of a redundant mechanism that is activated by both CTLA-4 and PD-1. These findings point out that targeting of PDCD4 could provide a potent strategy to improve anti-tumor immunotherapy.

P.B4.01.10

Differentiation of DBC T cells in the tumor microenvironment

A. Moustaki1, S. Ali1, B. Youngblood1
St Jude Children's Research Hospital, Memphis, United States.

Introduction: Differentiation of DBC T cells to effector, memory, or exhausted state has been well characterized during viral infection, but is poorly understood during tumorigenesis. Tumor infiltrating DBC T cells reside in and are adapted to a unique, cellularly diverse and rapidly evolving environment, which delivers highly immunosuppressive signals. Here we investigate the role of tumor microenvironment in DBC T cell activation in response to tumor antigens.

Methods: To monitor activation of tumor-specific DBC T cells, we stably expressed the dominant MHCI-restricted LCMV-derived epitope GP33, in MC38 tumor cells. Co-expression of a reporter gene encoding for mCherry allows for detection of antigen presentation.

Results: We have set up a model system of adoptive T cell transfer in order to elucidate mechanisms of T cell exhaustion and of ways to disinhume intratumoral T cells. Among these, we have identified a new functional role of induced regulatory T cells (iTreg) on our CTL, antigen-specific iTreg were co-transferred. Beside therapeutic response, ex vivo parameters were characterized, such as expression of co-inhibitory receptors (PDL-1, TIM-3, LAG-3, CTLA-4), Lamp-1-associated degranulation and intracellular production of IFN-γ and TNF.

Conclusions: Our findings highlight the importance of antigen and TME in the suppression of DBC T cells function. We also identify tumor associated monocytes/macrophages as a potential driver of antigen-induced DBC T cell exhaustion.
Poster Presentations

P.B4.01.11

Immunotherapeutic CD4+ T cells with tissue-resident features resist exhaustion in non-small cell lung cancer

A. E. Oji1, B. Piet1, D. van der Zwan1, H. Blaauwgeers1, M. Sundquist1, R. Stark1, J. Borst1, M. Nolle2, R. van Lier1, P. Homming1

1Sanquin Research, Amsterdam, Netherlands; 2QUV, Amsterdam, Netherlands; 3The Netherlands Cancer Institute, Amsterdam, Netherlands.

Resident memory T cells (TRM) inhabit peripheral tissues and are critical for protection against localized infections. Recently, it has become evident that CD103 expressing TRM are not only important in combating secondary infections, but also for the elimination of tumor cells. In several solid cancers, intratumoral CD103+CD8+ tumor infiltrating lymphocytes (TILs), with Treg properties, are a positive prognostic marker. To better understand the role of T RM in tumors, we performed a detailed characterization of CD8+ and CD4+ TIL phenotypes and functions in non-small cell lung cancer (NSCLC). We found comparable levels of CD8+ and CD4+ T cells infiltrates in tumors, but observed a sharp contrast in T RM, rats compared to surrounding lung tissue. While CD103+ CD8+ T cells were enriched in tumors, CD103+CD4+ T cell frequencies were decreased when compared to surrounding lung tissue. In line with the immunohistochemical analysis of the tumor microenvironment, CD8+ and CD4+ TILs expressed high levels of co-inhibitory receptors, such as PD-1, CTLA4, and PD-1, with the highest levels found on CD103 expressing TILs. Yet, CD103+CD4+ T cells proved to be the most potent producers of TNF-α and IFN-γ, while other CD4+ subsets and the majority of CD8+ TILs lacked such cytokine production. Furthermore, we found TILs with resident-memory features to express more costimulatory receptors, CD27 and CD28, when compared to lung T RM suggesting a less differentiated phenotype. Agonistic triggering of these receptors improved cytokine production of TILs. Our findings provide a rationale to target CD103+CD4+ TILs to improve the efficacy of immunotherapies and cancer vaccines.

P.B4.01.12

Identification of potential immunological markers associated with the PD1 checkpoint blockade Nivolumab in non small cell lung cancer patients

S. Ottone2, P. Carrega1, J. Cassoli2, C. Genovaz1, V. Fontanini3, F. Grassi1, L. Moretti1, M. Mingari1, G. Pietra1

1Center of Excellence for Biomedical Research, Department of Experimental Medicine University of Genova, Genoa, Italy; 2Laboratory of Immunology and Biotherapy, Department of Experimental Medicine University of Genova, Genoa, Italy; 3Surgical Unit, San Martino Hospital, Genoa, Italy; 4Clinical Epidemiology Unit, San Martino Hospital, Genova, Italy; 5Immunology Area, Pediatric Hospital Bambino Gesù, Rome, Italy; 6Center of Excellence for Biomedical Research, Department of Experimental Medicine University of Genova, Immunology Unit, San Martino Hospital, Immunology University of Genova, San Martino Hospital, Genoa, Italy.

PD-1 blockade represents a breakthrough in NSCLC therapy. However, to date robust biomarkers associated with response have not been identified. In this study we assessed whether particular immunological signatures evaluated over the course of treatment might be predictive of response to Nivolumab. We performed a deep phenotypic analysis in longitudinal blood samples of 73 advanced NSCLC patients treated with Nivolumab, collected at baseline and every 2 weeks before each therapy cycle. We examined the frequencies of several peripheral blood lymphocyte subpopulations (CD4+ T cells, CD8+ T cells, exhausted CD8+ T cells, NK cells) and their PD-1 expression. Cytometry data were then correlated with overall survival (OS) and clinical response as assessed by following RECIST. The higher % of CD3+ T cells at baseline correlated with longer OS (P =0.048). In contrast, a baseline higher % of PD-1+ CD3+ T cells (P =0.048), as well as of PD-1+ CD8+ T cells correlated with shorter OS (P=0.034). We next evaluated the frequency and the kinetics of exhausted CD8+ T cells on therapy. Interestingly, we observed a lower frequency of exhausted CD8+ T cells in pretreatment samples of PR/SD patients compared with PD patients (P=0.0462). In addition, in PR/SD patients, the frequency of exhausted CD8+ T cells was reduced during treatment (from cycle 1 to 4) (P=0.0001, P=0.0032, and P=0.0239, respectively). Our results suggest that a higher % of PD-1+ T cells at baseline could correlate with shorter OS. Furthermore, the different amount of exhausted CD8+ T cells could predict response and clinical outcome.

P.B4.01.13

Evaluation of potential factors contributing to the exhaustion of T lymphocytes in the tumor microenvironment

M. Rauatou1, A. Laermans1, N. Li2, J. Kim3, X. Ma3, E. Park4

1Advanced Cell Diagnostics, Newark, United States.

Immunosuppressive molecules and cells in the tumor microenvironment can lead to T cell dysfunction. CD8-positive cytotoxic T cells are ineffective in killing tumor cells primarily due to upregulated expression of inhibitory checkpoint molecules and decreased production of cytokines. Moreover, immune suppressive cell types and tumor associated macrophages are recruited to the TME, further establishing a suppressive immune environment. We evaluated expression profiles of immunosuppressive molecules and cells by applying RNAseq® assay and dual iSH-IRC staining. We evaluated CD8-positive cell infiltration in TME of human tissues from NSCLC and ovarian cancer. Selected tissues with either high or low CD8-positive cell number were evaluated for (1) the presence of Tregs and TAMs, (2) the expression of immune checkpoint molecules PD1, PD-L1, TIM3, and LAG3, (3) the expression of immune suppressors IDO1 and TGFB, and (4) IFNy expression in CD8-positive subsets. CD8-high tissues expressed higher level of immune checkpoint molecules, whereas CD8-low tissues exhibited an immune suppressive TME. In contrast, IDO1 and TGFB were expressed in tumors. CD8 cells were targeted in lower level and higher rate of immune response. Expression of IDO1 and TGFB was observed in tumor cells. This study highlights the potential of RNAseq® to understand the mechanisms associated with T cell dysfunction and exhaustion in TME. The RNAseq® platform is well suited for evaluating secreted factors in a cell type-specific manner and may facilitate the identification of biomarkers to stratify patients based on their specific cell state.

P.B4.01.14

HLA-DR in cytotoxic and regulatory T cells dictates breast cancer aggressiveness and patient response to neoadjuvant chemotherapy

D. R. Saravol1, S. Braga1,2, A. Jacinto2, M. G. Cabral1

1CEDOC, NOVA Medical School, Lisbon, Portugal; 2Instituto CUF de Oncologia, Lisbon, Portugal.

Neoadjuvant chemotherapy (NACT) is an integral component of the therapy for locally advanced breast cancer (BC). However, approximately half of the patients have no response. To promptly direct non-responders to personalized therapies, an urgent need to find a predictive biomarker of response. Tumor infiltrating lymphocytes are being appointed as biomarkers; nonetheless, tumor cells can escape the immune system, dampening CD8+ T cells (CTLs) and increasing regulatory T cells (Tregs). HLA-DR, a T cell activation marker, can rank CD8+ T cell aggressiveness in tumors, we performed a detailed characterization of CD8+ T cells at baseline correlated with longer OS (P =0.048). In contrast, a baseline higher % of PD-1+ CD3+ T cells (P =0.048), as well as of PD-1+ CD8+ T cells correlated with shorter OS (P=0.034). We next evaluated the frequency and the kinetics of exhausted CD8+ T cells on therapy. Interestingly, we observed a lower frequency of exhausted CD8+ T cells in pretreatment samples of PR/SD patients compared with PD patients (P=0.0462). In addition, in PR/SD patients, the frequency of exhausted CD8+ T cells was reduced during treatment (from cycle 1 to 4) (P=0.0001, P=0.0032, and P=0.0239, respectively). Our results suggest that a higher % of PD-1+ T cells at baseline could correlate with shorter OS. Furthermore, the different amount of exhausted CD8+ T cells could predict response and clinical outcome.

P.B4.01.15

Tumor-infiltrating Mucosa-Associated Invariant T (MAIT) cells express cytotoxic effector molecules and kill target cells

P. Sundström1, J. Siepzen1, F. Ahlmann1, M. Sundquist1, J. S. Wong1,2

1Dept of Microbiology and Immunology, Sahlgrenska Academy at University of Gothenburg, Sweden; 2Laboratory of Immunology and Biotherapy, Department of Clinical Immunology and Oncology, University of Gothenburg, Gothenburg, Sweden.

Tumor-infiltrating MAIT cells (TACs) express a transmembrane receptor recognizing microbial metabolites present on the MHC class I-like molecule MR1. Upon activation, they rapidly secrete cytokines and increase their cytotoxic potential. We showed recently that MAIT cells accumulate in human colon adenocarcinomas, but that their ability to produce IFN-γ upon polyclonal stimulation is compromised. Here, we investigated the cytotoxic potential of MAIT cells in colon adenocarcinoma, and to what extent it may be affected by the tumor microenvironment. MAIT cells were identified by flow cytometry and analyzed for their expression of cytotoxic effector molecules and degranulation. Polyclonal, T cell receptor-, and cytokine-mediated activation of MAIT cells from tumors induced increased Granzyme B, while degranulation was mainly seen in response to cognate antigen recognition. The cytotoxic potential of tumor-associated MAIT cells was similar to that of MAIT cells from unaffected colon. Furthermore, tumor infiltrating pre-activated MAIT cells killed antigen-presented targets presented by immunofluorescence or in direct contact with tumor cells in sections of colon cancer specimens. Taken together, our data demonstrate that tumor-associated MAIT cells from colon tumors have potent cytotoxic function and are not compromised in this regard compared to MAIT cells from the unaffected colon. We conclude that MAIT cells may significantly contribute to the protective immune response to tumors, both by secretion of Th1-associated cytokines and by direct killing of tumor cells.

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POSTER PRESENTATIONS

P.B4.01.16
A novel CD3/BCMA bispecific antibody for the treatment of multiple myeloma and plasma cell disorders selectively activates effector T cells with improved safety
M. Farinacci1,2,3, K. Junke1,2, R. Bueto4, V. van Schooten1, R. Bueto4, H. Voelz4

1Core Unit Immunocheck, Charité University of Medicine, Berlin, Germany, 2Institute for Medical Immunology, Charite University of Medicine, Berlin, Germany, 3B-Brandenburg Center for Regenerative Therapies, Charite University of Medicine, Berlin, Germany, 4Innoecbo Inc., Menlo Park, United States.

Introduction: Multiple myeloma (MM) is a cancer of plasma cells, and the second most common hematological malignancy. A promising therapeutic in development for MM is TNB-338B, an antiCD3/BCMA bispecific T-cell engager (BiTE). This T cell engager combines high tumor specificity (anti-BCMA arm) with a selective novel T cell activating CD3- arm. Efficacious T cell engagements with limited toxicity will allow combination therapies with other immune-modulators in MM and expansion into non-malign “plasma cell” disorders such as several autoimmune diseases.

Material and methods: TNB-338B was tested in standard in vitro and in vivo tumor models. To assess the potential risk of cytokine release syndrome (CRS) human peripheral blood mononuclear cells from healthy volunteers (n=3) were pre-incubated for 48 hours at high densities and, subsequently, incubated with increasing concentrations of TNB-338B overnight in the presence of recombinant BCMA protein coated to plastic. OKT3 and a T cell engaging bispecific with a high-affinity anti-CD3 arm were used throughout these experiments as positive controls.

Results: Both TNB-338B and the positive control T cell engager (PC) mediated tumor cell lysis in vitro and animal tumor models. In vitro analysis of upregulation of CD69 and CD137 on T cells showed that TNB-338B, in contrast to PC, preferentially activated CD4+ and CD8+ effector cells relative to CD4- T regulatory cells. Remarkably, T-cell activation by TNB-338B was only observed with low cytokine production compared to PC.

Conclusions: TNB-338B represents a novel immunotherapeutic with potentially improved safety and efficacy for the treatment of MM and pathogenic long-lived plasma cells.

P.B4.01.17
Prognostic implications of tissue resident memory T cells in human melanoma development
M. Willemsen1,2,3, G. Krebbers1,2,3, F. R. Kasiem1, T. R. Mato5,4, R. Luiten1,2,3

1Academic Medical Center, Amsterdam, Netherlands, 2Center for Cancer Center Amsterdam, Amsterdam, Netherlands, 3Amsterdam Infection & Immunity Institute, Amsterdam, Netherlands.

Tissue-resident memory T (T RM) cells permanently reside in epithelial barrier tissues and can respond rapidly upon reinfection. Recently, expression of the retention integrin very late antigen (VLA)-1, by vaccine-induced T cells was found to correlate with longer patient survival in melanoma. Interestingly, VLA-1 was frequently co-expressed with tissue resident memory markers CD69 and CD103. Furthermore, CD103-dependent T RM cells seem to play a key role in sustaining immunity to melanoma, indicating a crucial function for T RM cells in antitumor immunity. Yet, its role in melanoma development remains unknown and might be relevant, for there are numerous neoplastic lesions in the skin that rarely become overt cancers. This research, therefore, aimed to identify the prognostic relevance of skin resident memory T cells in human melanogenesis.

Healthy skin, naevocellulares nevi, dysplastic nevi, lentigo maligna, lentigo maligna melanoma, nodular primary melanoma, superficially spreading primary melanoma and cutaneous metastatic melanoma (all n=7) were analyzed by immunohistochemistry for the presence of T RM cells. The prognostic significance of CD69 and VLA-1 expression on T RM cells was also investigated.

The presence of T RM cells may serve as prognostic marker for disease progression. Furthermore, intratumoral T RM cells, especially in primary melanoma, may potentially be a good effector in cancer immunotherapies.

P.B4.01.18
General outlook of the immune surveillance status in oncological patients
P. Yolovlev1, D. Klyuyvyn1

1O.Bogomolets National Medical University, Kyiv, Ukraine, 2Taras Shevchenko National University of Kyiv, Kyiv, Ukraine.

Cancer initiation, progression and immune surveillance recruit lymphocytes as common key cellular players. The progression of the cancer occurs against the immunocompromised status in the patient. Much attention is being paid to the cellular branch of immunity in cancer patients either as marker or therapeutic target. Aim. To analyze the status of the immune surveillance system in patients with urinary tract cancer. Materials and methods. The retrospective analysis of pretreatment immunogram of 90 patients staged I-IV cancer of urinary bladder, kidney and prostate scheduled for definitive surgical treatment at the department of urology in 2015-2018 was performed. Levels of lymphocytes CD3+, CD4+, CD8+, CD3+56+16+, CD56-16+, CD19+, CD14+, and immune-regulatory index were compared to normal range disregard of the cancer stage. Results. Majority of immune cell parameters fell below the normal range: CD14+ were lower in 80% of patients, CD19+ in 76%, CD4+ in 42%, CD3+ in 40%, CD3+56+16+ in 20%, CD8+ in 15%, CD56-16+ in 12%, and IRI - in 40%. Elevation in immune cells content was observed: with CD3+56+16+ in 15%, CD8+ in 12%, and CD3+56-16+ in 12% of patients, mostly in advanced cancer patients. Conclusion. Vast deficiency in B-cell immunity was observed in majority (76%) of urological cancer patients, and prevails such of the T-cell branch. The T cells quantitative fluctuations present the diverse pattern, with elevation above normal range of cytotoxic, NK-cells, and T-suppressors mostly in patients with advanced urinary cancer.

P.B4.01.19
Evaluation of autologous tumor-antigen specific CD8+ T-cell responses to BRAFmut melanoma cells in the course of MAPKi treatment
N. Pieper1, F. N. Harbers1, A. Zaremba2, M. Schwamborn3, S. Lübeck4, B. Schrörs1, A. Sucker1, V. Lennerz1, T. Wölfl5, D. Schadendorf6, A. Pachser7, F. Zhao8

1University Hospital Essen, Essen, Germany, 2University Medical Center, Johannes Gutenberg University Mainz, Mainz, Germany.

Expressed in approximately 50% of melanomas, the V600 mutation in the BRAF gene promotes tumor proliferation via constitutive activation of BRAF-MEK-ERK in the MAPK signaling pathway. Significant clinical responses in patients with advanced metastatic melanoma can be achieved by targeting this axis using MAPK inhibitors (MAPKi), such as BRAFi and MEKi as single agents. The present study aimed to extensively investigate the relationship between tumor antigen-specific T cell responses and disease progression in BRAFmut melanoma patients under BRAFi treatment. Patients and methods: Tumor-antigen specific CD8+ T cells were identified by tetramer staining and intracellular cytokine staining (IFNγ) with or without MEKi supplementation. Only tumor antigen-specific CD8+ T cell responses with an IFNγ response ≥ 3% were evaluated. Our measurements demonstrated that the majority of antitumor CD8+ T cell responses were tumor antigen-specific and MEKi induced a significant increase in tumor antigen-specific CD8+ T cell responses. Conclusion. BRAFi treatment is associated with activation of specific tumor antigen-specific CD8+ T cell responses.

P.B4.01.20
Multifactorial contribution to follicular helper bias in the CD4 T cell response to a retroviral antigen
L. Danelli, G. Kassiottis

The Francis Crick Institute, London, United Kingdom.

The extent of CD4 T cell differentiation during an immune response is influenced by multiple intrinsic and extrinsic factors resulting in the generation of a diverse array of T helper (Th) cell phenotypes. Here, we analysed the response of CD4 T cells reactive with a murine retroviral envelope glycoprotein model antigen and identified an uncommonly strong bias towards follicular helper (Th) cell commitment and impaired Th1 differentiation in its natural context during retroviral infection. Comprehensive quantitation of variables known to influence CD4 T cell fate decision suggested that Th1 differentiation was directly correlated with increased Th2 cytokine production irrespective of Th2 cloneotypic avidity. However, strong Th2 cytokine signaling leading to Th2 downregulation and induction of LAG3 expression further restrained CD4 T cell commitment and differentiation. The response to the same antigen in the context of altered lymphocyte alteration and in different immunisation regimens generated a variable balance of Th1 and Th1 cells, suggesting that the stunted Th1 differentiation could be due to limited IL-2 availability during retroviral infection. These results highlight the potent contribution of T cell extrinsic variables in determining the relative balance of Th1 and Th2 responses according to the nature of the antigenic stimulus.

P.B4.01.21
Control of cytolytic lymphocyte anti-tumor functions through CD226 expression
M. Wuehrerse, A. C. Pichler, M. Joubert, H. Avet-Laloue, L. Martinet

Cancer Research Center of Toulouse, Toulouse, France.

Although CD8+ T lymphocytes activation mainly relies on TCR recognition of antigenic peptides presented by MHC-I, numerous signals transmitted through activation and inhibitory receptor interactions influence their functions. Promising results obtained by immune checkpoint blockers in cancer highlight the necessity of defining receptors that control cytotoxic lymphocyte anti-tumor reactivity. CD226 (DNAM-1) is an adhesion molecule stimulating CD8+ T cell cytotoxicity against target cells expressing its ligands, CD112 and CD135. Despite the recent defects associated with the absence of this molecule in several malignancies, the molecular role of CD226 in cytotoxic T lymphocyte biology remains poorly understood to date. We recently identified two distinct CD8+ T cells populations based on CD226 expression. We found that unlike the CD226+ counterparts, the CD226- T cells population has functional defects in response to TCR stimulation including lower proliferation, pro-inflammatory cytokine production and cytolytic activity. Global transcriptional analysis and gene transfer experiments revealed that CD226- T cells effector program initiated upon TCR stimulation requires CD226 expression.

P.B4.02 T-cell activation and exhaustion - Part 2

P.B4.02.01 Flow cytometry as a diagnostic tool to detect cellular and functional defects in patients with primary immunodeficiencies

M. Bitar, A. Balti, U. Sack; Institute of Clinical Immunology, Leipzig, Germany.

Primary immunodeficiency diseases (PIDs) are genetic disorders, that mostly cause susceptibility to infections and are sometimes associated with autoimmune and malignant diseases. Mutations can affect cells and molecules of the innate as well as the adaptive immune system (T and/or B cells). Here, we present the use of flow cytometry in the diagnostic cascade to identify possible PID. First, simple immunophenotyping of B-, NK-, CD4+, CD8+ double negative T cells and analysis of HLA-DR/CD38 is recommended to identify severe abnormalities in lymphocyte subsets e.g. in Bruton's Disease or SCID. Further differentiation (naïve, memory, recent thymic emigrants, activation markers etc.) of T, B- and NK-cell subsets in a second step is necessary to differentiate PID with defects in the subsets such as CVID or DiGeorge syndrome and many others. Based on that well defined immunophenotyping, functional analysis of leukocytes can be performed in a third step. For instance, flow cytometry is a useful tool for measurement of (1) T- and B-cell proliferation in case of different forms of lymphopoenia or unclear deficiencies in lymphocyte subsets, (2) phagocytosis/oxidative burst in chronic granulomatous disease. (3) loss-of-function STAT1 mutation and chronic mucocutaneous candidiasis CMC (gain-of-function gene mutations in STAT1), (4) STAT3 phosphorylation to distinguish between different Hyper-IgE syndrome (HIES) traits (autosomal dominant with STAT3 mutation in contrast to autosomal-recessive without STAT3 mutation). In conclusion, flow cytometry is well-standardized and a flexible method for investigation of most relevant leukocyte subsets and their immune function. Therefore, FCM should be considered as an important tool in PID-diagnostics.

P.B4.02.03 Function of T-bet and Eomes in CTLs - an evolutionary approach

1Institute of Medical Microbiology and Hygiene, University Medical Center Freiburg, Freiburg, Germany, 2Institute of Medical Microbiology and Hygiene, Department of Immunology, Freiburg, Germany, J. Ill Roberts Institute for Research in Inflammatory Bowel Disease, Cornell University, New York, United States, 3Institute of Experimental and Clinical Pharmacology and Toxicology, Freiburg, Germany.

The two T-box transcription factors Tbx21 (encoding for T-bet) and Eomesodermin (encoding for Eomes) have arisen from duplication of a single ancestral gene. In contrast to their ancestor both have variable functions in adaptive immunity indicating a benefit for the host throughout evolution. Usage of knock-out mice for T-bet or Eomes revealed redundant and non-redundant functions in T cells. However, this study emphasized that transcription factors act in highly context and cell specific networks. To dissect the individual contributions of T-bet and Eomes to the differentiation of T cells we developed a novel mouse model. We used TALEN technology to knock-in Eomesodermin into the Tbx21 locus to ensure the expression of Eomesodermin under complete transcriptional control of Tbx21. By infecting Tbx211/1 mice with 200 pfu LCMV WE we use FACS technology to compare transcriptional activities of Tbx21 and Eomesodermin on the protein level in T cells at day 8 and 30 post infection. We point out that T-bet is essential for expansion and cytotoxicity of CTLs and that Eomes compensates poorly for T-bet on an overall level in T cells. In conclusion our data shed light on the evolutionary pressure leading to the genesis of T-bet as a master regulator of type 1 immune responses.

P.B4.02.04 Effect of transition metal containing particulate matter (TMCPM) on the activity of human immune cells

A. Gałuszka, M. Stec, M. Siedlar, J. Baran; Department of Clinical Immunology, Institute of Pediatrics, Jagiellonian University Medical College, Cracow, Poland.

T-cell activation can be effectively regulated through the TCR receptor (TCR), engagement of costimulatory molecules, and cytokines. T cells can also directly recognize pathogens associated molecular patterns through the expression of toll-like receptors (TLRs). Whether TLR ligands can provide costimulatory signals and enhance antigen-driven T cell activation is not well understood. Here, we show that TLR2 and TLR7 ligands potentially lower the antigen threshold for cytokine production in T cells. To investigate how TLR triggering supports cytokine production, we adapted the protocol for flow cytometry-based fluorescence in situ hybridization (Flow-FISH) to mouse T cells. The simultaneous detection of cytokine mRNA and protein with single-cell resolution revealed that TLR triggering primarily drives de novo mRNA transcription. Ifng mRNA stabilization only occurs when the TCR is engaged. TLR-2 but not TLR7-mediated costimulation can potentiate mRNA stability at low antigen levels. Importantly, TLR2 costimulation also increases the percentage of polyfunctional T cells. In conclusion, TLR-mediated costimulation effectively supports and potentiates T cell effector function.

P.B4.02.05 Regulation of innate-like cell activation by IL-32

J. P. Hoff1, K. Powell1, H. Mehta1, P. Phalora1, T. Leng1, F. M. Buffet2, P. Klenerman11, C. B. Willberg11; 1The Peter Medawar Building for Pathogen Research, University of Oxford, Oxford, United Kingdom, 2Computational Biology and Integrative Genomics, Department of Oncology, University of Oxford, Oxford, United Kingdom, 3NHRI Biomedical Research Centre, University of Oxford, Oxford, United Kingdom.

Natural killer (NK) and innate-like T cells, including the mucosal-associated invariable T (MAIT) cell subset, share the ability to respond to cytokines such as IL-12 and IL-18. A novel pluriptotent cytokine, IL-32, has been reported to mediate NK cell responses to cytokine-induced activation, but its effect on MAIT cells is currently unknown. We show IL-32 can significantly increase cytokine-mediated IFN-γ and Granzyme B expression in CD161+ T cells, which includes the MAIT cell subset, within PBMCs and isolated CD8+ T cells. However, it does not have any measurable effect on TNFα-expression. Moreover, IL-32 promotes cytokine-mediated upregulation of checkpoint markers, including LAG3, PD1 and TIM3. The observed IL-32-mediated effects are time- and dose-dependent. No significant effect of IL-32 on TCR-mediated responses was detected. Blocking IL-32 during cytokine- or T-cell-mediated MAIT cell activation, within PBMCs, decreased MAIT cell responses significantly, indicating a crucial role for IL-32 in MAIT cell activation. Furthermore, IL-12+IL-18 stimulation of PBMCs and CD161+ T cells resulted in the expression of IL-32 by CD161+ T cells, including the MAIT cell subset. These cells are also the predominant subset of T cells expressing IL-32 in response to IL-12 and IL-18, indicating a potential feedback loop. Our findings broaden the understanding of cytokine- and bacterial-mediated activation of MAIT cells and potentially provide another reference point for future clinical intervention in disease.
POSTER PRESENTATIONS

P.B4.02.07 Acid Sphingomyelinase deficiency reduces cytotoxic activity of CD8+ T cells during melanoma progression
M. Hose1, A. Westendorf2, J. Bueter3, A. Haimovitz-Friedman4, W. Hansen5; 1Institute of medical microbiology, University Hospital Essen, Germany; 2Memorial Sloan Kettering Cancer Center, New York City, United States. Acid sphingomyelinase (Asm) is a hydrolyzing enzyme and part of the sphingolipid metabolism. After activation Asm converts sphingomyelin to ceramide at the outer leaflet of the plasmamembrane. Self-accumulating properties of ceramide lead to the generation of ceramide-enriched platforms. These platforms are involved in receptor clustering and thereby in intracellular signal transduction. Tumor cells were shown to have a double growth rate when implanted into Asm-deficient mice as compared to wildtype mice. The research suggests that melanoma cells possess specific mechanisms to circumvent Asm deficiency. Furthermore, the data indicate that ceramide accumulation in Asm-deficient cells may have implications for tumor cell survival and proliferation. Future studies are needed to elucidate the exact mechanisms underlying the observed phenotypes.

P.B4.02.08 Analysis of signaling pathways triggered by costimulatory receptor GITR
Š. Janušová, H. Draberová, A. Drabek, O. Stepanek, P. Draber; Institute of molecular genetics, Prague, Czech Republic. T cell immune responses are initiated upon the recognition of an antigen presented on MHC glycoproteins by specific T cell receptors. However, for proper immune response, additional costimulatory signals are required. Several members of the TNF-receptor superfamily, such as GITR, CD27, CD137 or OX40, can provide the required costimulatory signal, which affects T cell survival, proliferation, and effector functions. These receptors are expressed on activated T cells and, after engagement with their ligands, they trigger downstream signaling events, such as activation of NF-κB, p38 or JNK signaling pathways. The precise understanding how the costimulatory signaling is propagated would provide a potential target(s) to modulate T cell responses in autoimmune diseases or cancer. In this study we analysed molecular mechanisms modulating signaling after GITR stimulation. We aimed to find novel molecules participating in these pathways and elucidate how they affect GITR-induced signaling responses.

P.B4.02.09 TCR and inflammatory signals tune human MAIT cells to exert specific tissue repair and effector functions
T. Leng, T. King, C. Willberg, P. Klenermann; Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom. Background: Human mucosal associated invariant T (MAIT) cells are present in circulation as well as immune privileged sites such as the liver and the gut. They are a homogenous intraepithelial lymphocyte subset and their phenotypic and functional properties. Studies have illustrated an array of MAIT cell effector functions, ranging from MR1-mediated antibacterial responses to TCR-independent, cytokine-mediated antiviral properties, also proinflammatory roles in autoimmune diseases. Aim: We aim to examine the impact of combinations of TCR-dependent and -independent signals in blood and tissue-derived human MAIT cells, and to extend our knowledge on the functional properties of this tissue-resident T cell subset. Conclusion: While TCR triggering is insufficient to drive full activation, gene expression signatures of TCR-triggered MAIT cells showed specific enrichment of tissue-repair functions (e.g. TRIF, FURIN, VEGFA, CSF1). Activation of certain effector and antimicrobial functions - measured by IFN-γ release and Granulyme B expression – may be triggered further with specific innate signals in blood and tissue, such as the TNF superfamily member TLLA. These features were reproduced in gut-derived cells. By genome-wide comparison of functional responses to TCR-dependent and independent signals, we defined novel functions of MAIT cells relevant not only to host defence but also tissue homoeostasis. Discussion: Our study suggests that MAIT cells could serve a potential therapeutic target in IBD treatment, where reducing local inflammation and promoting tissue repair is essential. We hope to gain deeper insight into relevant functions of MAIT cells by investigating transcriptional signatures of gut-derived MAIT cells.

P.B4.02.10 A segment of amino acids of LAT adaptor modifies its turnover and has a dual role in TCR intracellular signaling
M. Martinez de Arbulu-Echevarria1, J. Narbono-Sanchez1, C. Fernandez-Ponce1, I. Vico-Barranco1, J. Muñoz-Miranda1, M. Rueda-Ygueravide1, M. L. Dustín2, A. Maiés3, M. Duran-Ruíz1, F. Garcia-Cazar1, A. Aguado-Vidal4; 1University of Gdansk, Gdansk, Poland; 2Neumology and Allergic diseases, Hospital Puerta del Mar, Cadiz, Spain; 3The University of Oxford, Headington, United Kingdom; 4Institute of Immunology and Experimental Therapy, Wrocław, Poland. Normal T cell development and function requires TCR/CD3 signaling that proceeds through the activation of protein tyrosine kinases and phosphorylation of downstream molecules. The membrane-anchored adaptor Linker for Activation of T cells (LAT) has an essential role transducing intracellular signals coming from the TCR/CD3 complex. It has been shown that upon T cell activation LAT interacts with the tyrosine kinase Lck, leading to the inhibition of its kinase activity. The segment comprising residues 112-126 of human LAT is required for its interaction with Lck, and this domain is rich in negatively charged amino acids and is conserved among different species. In order to deepen into the functional relevance of LAT-Lck interaction, we have substituted this segment of LAT with a non-charged segment of amino acids and expressed this mutant LAT (LAT-NIL, from Not Interacting with Lck) in LAT-deficient JCaM2 cells. Substitution of this segment in LAT does not alter its expression in the plasma membrane, but prevented the activation-induced interaction with Lck. LAT-NIL mutant showed a reduced stability with regard to WT-LAT after cycloheximide treatment. JCaM2 cells expressing this mutant form of LAT showed a statistically significant higher phosphorylation of LAT in tyrosine residues 171 and 191, and also enhanced ZAP70 phosphorylation approaching borderline statistical significance (p=0.051). Nevertheless, downstream signals such as Ca2+ influx or MAPK pathways were partially inhibited. Overall, our data reveal that LAT-Lck interaction constitutes a key element regulating proximal intracellular signals coming from the TCR/CD3 complex.

P.B4.02.11 Cell type specific activation of Mucosal associated invariant T cells in the Liver
H. K. Mehta1, C. B. Willberg2, P. Klenerman3; 1University of Oxford, Oxfordshire, United Kingdom, 2Petrer Medawar Building for Pathogen Research, University of Oxford, Oxfordshire, United Kingdom, 3Oxford NIHR Biomedical Research Centre, The John Radcliffe Hospital, Oxfordshire, United Kingdom. Mucosal associated invariant T (MAIT) cells are enriched within the liver representing a major hepatic T cell subset. However, the ability of diverse liver parenchymal cells to interact with MAIT cells has not been well explored. We investigated the capacity of different hepatic cell lines to activate MAIT cells in response to both bacteria and viruses. Immortalized primary hepatic epithelioid (IHP) (HHL12), hepatocellular carcinoma (HCC) (HepG2 and Huh7), stellate (TW-NT) and liver sinusoidal endothelial cell (LSEC) lines were used to stimulate MAIT cells in the presence of E or hepatitis viruses. MAIT cell activation was analysed by flow cytometry. The HCC lines, HepG2, Huh7 and VPH line, HHL12 and stellate cell lines were not able to stimulate MAIT cells after exposure to E.coli or viruses. However, the LSEC cell line is able to readily activate MAIT cells for cytokine production (IFN-γ and TNF-α). In addition to this we observed an increase in Grb production from MAIT cells upon activation. LSECs activate MAIT cells primarily through the MRL pathway but also utilise the alternative cytokine pathway (IL-12/IL-18). HBV-infected LSECs partially activated specific MAIT cells. In conclusion, through their anatomical location and interaction with pathogens, together with their functional activity as antigen presenting cells, LSECs may be critical activators of MAIT cells in the liver for bacterial and potentially for viral infections.

P.B4.02.12 Differences in ERAP1 allelic function correlate with HPV epitope processing, TIL status and prognosis in HPV-positive OPSCC
E. Reeves, E. King, G. Thomas, T. Elliott, E. James; University of Southampton, Southampton, United Kingdom. Human papillomavirus (HPV) infection accounts for 5% cancers worldwide, with cervical carcinoma and oropharyngeal squamous cell carcinoma (OPSCC) being most common. Levels of tumour infiltrating lymphocytes (TIL) in HPV+ cancers are an indicator of prognosis and survival, with fewer TIL having a worse clinical outcome. Polymorphisms in the endoplasmic reticulum aminopeptidase1 (ERAP1), a key component of antigen processing trimming N-terminally extended peptides for MHC I, are associated with prognosis and outcome in cervical carcinoma. We have previously demonstrated ERAP1 to be highly polymorphic, forming distinct alleles with functional differences. Here we investigate the contribution of ERAP1 in OPSCC. We identified a number of ERAP1 allelic combinations (co-dominantly expressed chromosomal copies) from HPV+ OPSCC patients. Assessment of trimming function revealed a range of abilities to generate the model epitope SINFEHL from individual amino acid precursors. Further analysis revealed that the activity of ERAP1 from TIL1 and TIL2 patients correlates with the property of the amino acid extension. Importantly, the ability of ERAP1 to generate the HPV-16 E7 epitope (LLMGTLGIV) from predicted precursors also correlated with TIL status. ERAP1 allelotype pairs identified in TIL1 patients were poor at generating the final epitope (SINFEHL and LLMGTLGIV), whereas those identified in TIL2 patients generated the epitopes efficiently. These data reveal that ERAP1 gene sequence and function stratifies with TIL levels and prognosis, suggesting that the successful presentation of HPV-16 epitopes at the cell surface, and thus a strong anti-HPV T cell response, depends on the ERAP1 allelotype pairs expressed within an individual.
Assessment of CD320 expression and vitamin B12 function on human T-cells
R. Schmidt, A. Doppler, M. Gerner, L. Ziegler, K. Schmetterer
Department of Laboratory Medicine, Vienna, Austria.

Background: Vitamin B12 is an essential co-factor for proliferating and metabolically active cells. After uptake into the organism, it is bound to transport proteins in the serum. Transcobalamin II is the transport protein that delivers Cobalamine to peripheral cells, where it binds to CD320, the Transcobalamin II receptor. Vitamin B12 deficiency presents with failure of highly proliferating and metabolically active tissue resulting in megaloblastic anemia or neurological dysfunctions. The effects of vitamin B12 on activation and function of lymphocytes have not been fully investigated so far.

Methods: T-cells were negatively isolated from peripheral blood derived PBMC and polyclonally stimulated using antiCD3/antiCD28 coated beads. Following activation, mRNA levels and protein concentration of CD320 were measured by immunoblotting and flow cytometry. For functional assays, binding of TCII to CD320 was blocked using a monoclonal antibody.

Results: Upon activation, CD320 levels were up-regulated, with the highest expression after 72h. In the presence of anti-TGI antibody, the proliferation of T-cells decreased while early activation markers, such as surface expression of CD69, CD25 and CD71 and production/secretion of IL-2 were not influenced. Similarly, TCR-proximal signaling events, such as phosphorylation of the CD3-zeta chain and the adapter molecules LAT and SLP-76 were also not influenced by the presence of anti-TCI.

Conclusion: These observations indicate that CD320 is an activation marker on T-cells. Furthermore, Vitamin B12 is essential for long-term T-cell activation/proliferation while activation steps preceding the induction of proliferation are independent of vitamin B12.

Assessment of CD320 expression and vitamin B12 function on human T-cells
P. B4.02.13

Molecular transport at the immunological synapse controls T-cells
M. van Ham1, N. Amberg1, L. Philipson2, S. Klische2, J. Niemz2, L. Simeon1, C. Falk1, A. Müller1, J. Hülken2, B. Schraven2, L. Jänsch2
1Heilmann-Research for Infection Research, Braunschweig, Germany, 2Otto von Guericke University, Magdeburg, Germany

We report an assembly and activity of the T-cell signalling network at the immunological synapse (IS) requires molecular transport of signalling microvesicles (MV). Following T-cell activation, MVs are rapidly recruited along IS-projecting microtubules by the dynein motor complex. Interestingly, molecular transport at the IS discriminates Tnun- and Treg cells. We reported a subset-specific TCR network assembly and identified involved cytoskeleton regulators by phosophorepoimics. Among those we identified the dynein light intermediate chain 1 (LIC1), a dynein motor subunit that regulates cargo selection.

To analyze the role of LIC1 in T-cell activation, component recruitment and IS formation we generated NL1-deficient Jurkat T-cells (LIC1−/−) cells. General T-cell functions including proliferation, adhesion, migration and TCR surface expression were not affected. Upon TCR engagement, however, LIC1−/− cells indicated aberrant phosphorylation dynamics at proximal components. Conjugation with Raji B cells revealed enhanced accumulation of an Lck phosphovariant at the IS. In addition, activated LIC1−/− cells exhibited reduced secretion of selected cytokines. Thus, the dynein motor subunit LIC1 seems to act as a TCR-dependent regulator for the selected adaptation of TCR signalling and cytokine release.

A better understanding of the regulatory network underlying MV molecular transport at the IS might pave the way for novel checkpoints to regulate TCR signalling of T-cell subsets in vitro and in vivo.

Role of diacylglycerol kinase alpha in X-linked lymphoproliferative disease 1 (XLP1) and autoimmunity
1Department of Translational Medicine, university of piemonte orientale, Novara, Italy, 2Institute for Research and Cure of Autoimmune Diseases, Novara, Italy, 3School of Medicine, University Vito e Salolute San Raffaele, Milan, Italy, 4Department of Pharmaceutical Science, University of Piemonte Orientale, Novara, Italy, 5Department of Health Sciences, School of Medicine, University Piemonte Orientale, Novara, Italy, 6Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, United States.

X-linked lymphoproliferative disease 1 (XLP1), a primary immunodeficiency due to mutations in SLAM adaptor protein (SAP). SAP deficiency results in constitutive diacylglycerol (DG) secretion of selected cytokines. Thus, the dynein motor subunit LIC1 seems to act as a TCR-dependent regulator for the selected adaptation of TCR signalling and cytokine release.

A better understanding of the regulatory network underlying MV molecular transport at the IS might pave the way for novel checkpoints to regulate TCR signalling of T-cell subsets in vitro and in vivo.

Role of diacylglycerol kinase alpha in X-linked lymphoproliferative disease 1 (XLP1) and autoimmunity
P. B4.02.16

Use of melatonin from yeast-like fungies Nadsoniella nigra sp. XI (J. Galindez, Antarctica) in surgically treated urological stage IV cancer patients
P. Yakolov1, F. Falalyeyeva2, L. Garmanchuk3, T. Beregov2, L. Ostapchenko2
1O.Bogomolets National Medical University, Kyiv, Ukraine, 2Department of Translational Medicine, university of piemonte orientale, Novara, Italy, 3Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, United States.

Nadsoniella nigra sp. XI (J. Galindez, Antarctica) in surgically treated urological stage IV cancer patients
P. B4.02.17
Deletion of Map4k4 induces CD8 T cell cytokine production, activation, proliferation and target killing

Map4k4 is a serine threonine kinase of the STE20 family that is expressed broadly. It is highly expressed in immune cells. We investigated the role of Map4k4 in CD8 T cell function. Deletion of Map4k4 induces CD8 T cell cytokine production, activation, proliferation and target killing in vitro. Map4k4 KO (induced knock-out) animals showed reduced tumor growth when MC38 cells were injected subcutaneously. In the tumors, T cells expressed less exhaustion markers and produced more inflammatory cytokines when stimulated ex vivo. We showed that CD8 T cells were both necessary and sufficient for reduced tumor growth difference in the Map4k4 KO animals. Using LCMV-Armstrong infection model, we showed that there is increased antigen specific CD8 T cells showed enhanced cytokine production. We also showed elevated activation markers using OT1 transgenic model. To sum up, Map4k4 knockout leads to enhanced CD8 T cell proliferation and activation. Mechanistically, we found that Map4k4 kinase activity is necessary for its role in CD8 T cells and it signals through ERK family of proteins.

P.B4.03 T-cell activation and exhaustion - Part 3

P.B4.03.01 Visualizing the immune response of the T helper cells to the osteopathic manipulative treatment in normal subjects

A. Abdeljalil, N. Abdeiandrof, S. Nasif;
Faculty of physical therapy - Cairo University, Cairo, Egypt.

Purpose: This study was designed to investigate the effect of selected osteopathic lymphatic techniques on immune system in healthy subjects. Method: Forty five subjects (33 males and 12 females), with age ranged from 20 to 30 years old participated in this study. They were assigned into three equal groups each one has 15 subjects: group A received sternal pump and sternal recall techniques. Group B received thoracic lymphatic pump and splenic pump techniques for 12 sessions, three sessions per week. Group C (control group) did not receive any physical therapy modality. Absolute count of CD4 and WBCs count were used to evaluate participants before and after the study. Results: Statistical analysis revealed that there was a significant increase in CD4 P value was 0.045 and WBCs count P value was 0.006 between before and after treatment with the second group in the two experimental groups. While there was no significant difference in the same measuring variables in the first and control groups. Comparison between groups revealed that there was a significant difference between the first and second groups in CD4, P: probability=0.05. Conclusion: The second osteopathic manipulative treatment group was the effective method of enhancing the immune system in healthy subjects (thoracic lymphatic pump (TLPT) and splenic pump techniques (SPT). Key words: Osteopathy, CD4, Thoracic lymphatic pump, splenic pump technique, Sternal pump technique and Sternal recall technique. Ethical committee approval: Cairo university.P.T.R.E/012/00945 ANZCTR NO: ACRTRN1261600216415

P.B4.03.02 The transcription factor TOX controls differentiation and maintenance of T cells in chronic infection

Technische Universität München (TUM), Freising, Germany.

Introduction: During chronic infections, virus-specific CD8 T cells acquire a differentiation program that is distinct from the one acquired in acute infections. In this case, the presence of activated T cells causes T cells to become dysfunctional which means that they show impaired effector functions and up-regulated inhibitory receptor expression. To understand the molecular foundation of CD8 T cell dysfunction, we compared the transcriptional profiles of normal and dysfunctional CD8 T cells. We found the transcription factor TOX as one of the most upregulated gene in chronically stimulated CD8 T cells. Material and Methods: To study the function of TOX, we generated a conditional KO mouse which facilitates peripheral deletion of functional TOX protein in P14 T cells. Following adoptive transfer of KO or WT P14 in naïve mice, we monitored their behavior in LCMV Armstrong or clone13 infections. Flow cytometry, NGS, immunofluorescence and immunochemistry analysis were performed. Results: The absence of TOX impaired the survival of dysfunctional CD8 T cells, without affecting CD8 T cells baring an acute phenotype. This impaired survival goes along with a loss of the critical TCF-1 expressing population, decreased PD-1 but increased KLRG1 expression and augmented cytokine production. The resulting enhanced effector phenotype leads to a better viral clearance but also to an increase in immunopathology. Conclusions: TOX promotes the dysfunctional phenotype in CD8 T cells, nonetheless ensures their fitness for long term maintenance during chronic infection.

P.B4.03.03 Innocent Bystanders: Chronic Virus Infection Compromises Memory Bystander T cell Maintenance and Function

‘ETH Zurich, Zurich, Switzerland, \‘University of Zurich, Zurich, Switzerland.

Chronic viral infections are widespread among humans, with approximately 8-12 chronic viral infections per individual and there is epidemiological proof that these impair heterologous immunity. We studied the impact of chronic virus infections on the phenotype and function of memory bystander CD8 T cells. Active chronic virus infection had a profound effect on total numbers, phenotype and function of memory bystander T cells in mice. The phenotypic changes included upregulation of markers commonly associated with effector and exhausted cells and were induced by IL-6 in a STAT1-dependent manner in the context of chronic virus infection. Furthermore, bystander CD8 T cell functions were reduced with respect to their ability to produce inflammatory cytokines and to undergo secondary expansion upon cognate antigen challenge with major cell-extrinsic contributions responsible for the diminished memory potential of bystander CD8 T cells. These findings open new perspectives for immunity and vaccination during chronic viral infections.
POSTER PRESENTATIONS

P.B4.03.04
Distinct function of the co-signaling partners BTLA and HVEM on the virus-specific CD8+ T cell responses during LCMV infection

P. Diethelm, J. Schmitz, J. Kieslow, M. Kopf;
Institute of Molecular Health Sciences, Zurich, Switzerland.

Activation of naive T cells is controlled through engagement of co-stimulatory and co-inhibitory molecules. It has been reported that binding of HVEM (Herpes Virus entry mediator) to BTLA (B and T lymphocyte attenuator) inhibits T cell expansion however recent evidence suggests that BTLA can serve reciprocally as an activating ligand for HVEM. By infecting single and double KO animals with an acute or a chronic dose of LCMV we made the unexpected finding that neither effector anti-viral T cell responses nor memory formation were impaired. Accordingly, no difference in virus clearance was found between knockout and control type animals. Interestingly, staining of BTLA and HVEM on gp33-specific CD8+ T cells at the peak of the immune response revealed that both molecules were down regulated implying that this may promote T cell expansion. Indeed, retroviral overexpression of HVEM on virus-specific CD8+ T cells resulted in reduced numbers of effector cells 7dpi. In contrast, BTLA overexpression rather increased the cytokine production of gp33-specific CD8+ T cells. Whether sustained expression of HVEM affects proliferation or survival of anti-viral CD8+ T cells during the effector phase and whether the cytotoxic activity of BTLA overexpressing CD8+ T cells is increased is currently under investigation. Together these data imply that HVEM down regulation on gp33-specific CD8+ T cells ensures efficient accumulation 7dpi and thus control of an LCMV infection whereas down regulation of BTLA dampens the cytokine response of anti-viral CD8+ T cell lines thus limits inflammation.

P.B4.03.05
Bacillus Calmette-Guerin (BCG) causes Vgamma9Vdelta2 T-cell proliferation and potentiates cytotoxic responses towards tumour cells

J. R. Fenn, R. Reljic, S. Sharpe, M. D. Bodman-Smith;
- St. George’s, University of London, London, United Kingdom; *Public Health England, Salisbury, United Kingdom.

Vγ9Vδ2 T cells can recognize malignantly transformed cells as well as those infected with mycobacteria. This cross-reactivity supports the idea of using mycobacteria to manipulate Vγ9Vδ2 T cells in cancer immunotherapy. To date, therapeutic interventions using Vγ9Vδ2 T cells in cancer have involved expanding these cells in or ex vivo using azole-based acid (2A). In this study we show that the mycobacterium-based vaccine, Bacillus Calmette-Guerin (BCG), also causes Vγ9Vδ2 T-cell expansion in vitro. We phenotypically and functionally compared Vγ9Vδ2 T-cells expanded using 2A to those expanded using BCG and compared the ability of these two populations of Vγ9Vδ2 T cells to kill myeloid THP-1 target cells. We show that 2A expanded Vγ9Vδ2 T cells kill THP-1 cells but are unable to target untreated THP-1 cells. Conversely, BCG expanded Vγ9Vδ2 T cells are able to target both BCG and 2A treated THP-1 cells. Furthermore, BCG expanded, but not 2A expanded, THP-1 cells whereas 2A expanded Vγ9Vδ2 T cells were not. These data suggest that BCG treatment could be a way of generating Vγ9Vδ2 T-cells with greater tumouricidal potential compared to 2A treatment and that either 2A or BCG could be used intratumourally as a means to potentiate stronger anti-tumour Vγ9Vδ2 T-cell responses.

P.B4.03.06
Molecular imaging of the antigen recognition dynamics in CD8+ cytotoxic T-cells

M. Kraller, R. Platzek, P. Specht, J. Huppa, H. Stockinger;
Medical University of Vienna, Vienna, Austria.

Cytotoxic T-cells (CTLs) can detect with their low affinity T-cell antigen receptors (TCRs) the presence of even a single antigenic peptide-loaded MHC molecule (pMHC) among thousands of structurally related yet non-stimulatory pMHCs (Purbhoo et al. 2004). How they achieve this is not clear but appears to depend at least in part on the special binding conditions within the special constraints of the immunological synapse, the area of contact between a T-cell and an antigen presenting cell. Here receptors and their ligands are not only pre-oriented, but are often enriched in specific membrane domains and also subjected to cellular forces. To relate these cell biological parameters to T-cell antigen sensitivity, we developed a novel experimental setup. It allows the use of molecular imaging modalities. We confront TCR transgenic CTLs with a glass-supported lipid bilayer (SLB) functionalized with pMHC, adhesion and co-stimulatory molecules. This allows us to conduct (single molecule) measurements in noise-attenuated Total Internal Reflection (TIRF) mode, to control for ligand composition and density to quantitate their specific influence on TCR-pMHC binding and TCR-proximal downstream signalling. We also plan to assess the role of CD8 co-receptor engagement with the use of pMHC mutants, which are deficient in CD8 binding. In this fashion we expect to gain novel insights into cell biological and molecular processes underlying the phenomenon of CTLs towards antigen.

P.B4.03.07
Analyzing chimeric single chain antigen receptors by using a triple parameter T cell reporter line

1 Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria; MRC Centre for Transplantation, King's College London, Guy's Hospital, NIHR Biomedical Research Centre, Guy's & St Thomas' NHS Foundation Trust & King's College London, Guy's Hospital, London, United Kingdom, *NIHR Biomedical Research Centre, Guy's & St Thomas' NHS Foundation Trust & King's College London, Guy's Hospital, London, United Kingdom, 1Department of Hematology, Leiden University Medical Center, 2300 RC Leiden, Netherlands, 2Department of Clinical Cell Biology and FAES Core Unit, Children's Cancer Research Institute (CCRI), Vienna, Austria.

Adaptive transfer of genetically modified T cells expressing single-chain antigen receptors like chimeric antigen receptors (CAR) or chimeric single-chain TCR (cTCR) are emerging and highly promising approaches for cancer treatment. Within this study different signaling domains for such receptors were assessed in a Jurkat triple parameter transcriptional reporter cell line 7E1 [Jurkat 7E1-TPR]. This T cell reporter line allows to simultaneously assess the activity of three transcription factors that play essential roles in T cell activation processes - NF-kappaB, NFAT and AP-1. A CAR recognizing HLA-A2 and the HLA-A2 restricted cTCR X15 specific for the human T cell lymphotropic virus (HTLV) Tax peptide were used as model single-chain antigen receptors. Both were fused to signaling domains derived from 4-1BB, CD28 and CD3z to generate signaling modules that correspond to first, second and third generation CARs. The resulting reporter lines were stimulated with engineered antigen-presenting cells (APC). Assessment of reporter gene expression by flow cytometry showed that activation of Jurkat reporter cells critically depended on the signaling modules contained in the single-chain antigen receptors. Direct comparison of 4-1BB and CD28 as costimulatory modules in both types of receptors (CAR and cTCR) demonstrated a much higher capacity of the 4-1BB domain to induce reporter gene expression. Our results indicate that transcriptional T cell reporter cells are a powerful means to assess signalling domains for single-chain antigen receptors.

P.B4.03.08
The role of Interleukin-18 on the phenotypical and functional plasticity of cytotoxic T cells in pancreatic cancer

Clinic for Gastroenterology, Endocrinology, Metabolism and Infectology, Philips-University Marburg, Marburg, Germany.

Introduction: CD8+ cytotoxic T cells (CTL) play a central role in tumor response mechanisms. In pancreatic cancer CTL show decreased activation. Mechanistically, proinflammatory cytokines, like IL-1 and IL-18, might play a crucial role in the induction of this dysfunctional state. This project investigates the role of IL-18 on cytotoxic T cell responses in a murine model of pancreatic cancer.

Methods: Antigen-specific CTL were generated from transgenic OT-I mice and from transgenic OT-I mice crossed with IL-18R-KO mice. These mice were infected with the GFP-expressing LCMV. We used the GFP signal to track the depletion of the CD8+ T cell population over time. We analyzed the CD8+ T cell population in the spleen and lymph nodes of these mice using flow cytometry.

Results: IL-18 enhanced cytokine production and increased cell proliferation of CTLs. IL-18 also increased the expression of adhesion molecules and co-stimulatory receptors on the surface of CTLs.

Conclusions: These results suggest that IL-18 plays a crucial role in the regulation of the functional plasticity of CTLs in pancreatic cancer. Further studies are needed to investigate the molecular mechanisms underlying these effects.

Bacillus Calmette-Guerin (BCG) could be used intratumourally as a means to potentiate stronger anti-tumour Vγ9Vδ2 T-cell responses.
Abstracts of the 5th European Congress on Immunology - ECI 2018 - Amsterdam, The Netherlands
A hallmark of chronic infections is exhausted CD8 T cells, characterized by a perturbed transcriptional program, upregulation of inhibitory receptors, impaired effector function, reduced numbers, and alteration of normal memory development, driven by prolonged T cell receptor (TCR) engagement. This project focuses on characterizing the kinetics of TCR signaling in different tissues in mice chronically infected with Lymphocytic Choriomeningitis virus. TCR signaling was quantified using in vivo expression of specific transgenic CD8 T cells expressing a reporter under the control of the Nur77 promoter. Our data reveals that, despite available antigen in vivo, Nur77 expression is very low in exhausted cells. In addition, these cells express multiple co-inhibitory receptors known to inhibit TCR signaling. Indeed, both blockade of the major inhibitory PD-1-PD-L1 pathway and transfer of target cells lacking ligands binding co-inhibitory receptors into chronically infected mice increased Nur77 reporter levels temporarily. Preliminary results of single-cell RNAseq performed on exhausted cells isolated from various organs revealed distinct subpopulations, suggesting that tissue microenvironment has a major impact on shaping the structure, phenotype, and function of these cells. Thus, persistent TCR triggering during chronic infection leads to a near complete shut-down of in vivo TCR signaling, largely due to the combined action of inhibitory receptors, explaining the poor ability of exhausted cells to kill endogenous virus-infected targets. However, cytotoxic potential is preserved because they can efficiently kill transferred target cells lacking ligands for inhibitory receptors. Moreover, single-cell transcriptionomics reveals that exhausted cells are heterogeneous and adopt tissue-specific signatures.

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**P.B4.03.14**

*In vivo TCR signaling during chronic viral infection*

L. Sandu, D. Cerletti, A. Oxenius, M. Claassen; ETH, Zürich, Switzerland.

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**P.B4.03.15**

Phenotypic and functional characteristics of HER2-specific cytotoxic T lymphocytes activated by DNA-transfected dendritic cells and enriched by magnet sorting and cytokine stimulation

M. Kuznetsova, J. Lapotnikova, S. Sennikov; Research Institute of Fundamental and Clinical immunology (RIFCI), Novosibirsk, Russian Federation.

Introduction. Approaches based on antigen presentation to cytotoxic T lymphocytes (CTLs) by dendritic cells (DCs) are actively applied in modern cancer immunotherapy. The aim of the study was to obtain CD8+ T cells specific for HER2/neu (HER2) antigen and evaluate their phenotype and effector function.

Methods. A developed protocol for obtaining HER2-specific CD8+ T lymphocytes was used, consisted of magnet-assisted transfection of monocyte-derived DCs, co-culturing of antigen-loaded DCs with autologous lymphocytes, magnet sorting of CTLs specific to HER2 epitopes and stimulation of separated CTLs by IL-2, IL-7 and IL-15. HER2-specific CTL phenotyping and identification of T memory subsets was carried out using multicolor flow cytometry. Cytotoxicity assay against HER2-expressing MCF-7 cell line was performed to evaluate effector function of obtained T cells.

Results. Here we show that HER2-specific CTL populations obtained with the protocol we developed contain large percent of T stem cell-like memory cells (TSCM) (about 60% of CD8+ HER2-specific T cell population). Cytotoxicity analysis of HER2-specific T-cells showed significantly higher level of cytotoxic effect against cell line MCF-7, as compared with that of the fraction of activated PBMCs and that of the fraction of CD8+ T cells of different specificities.

Conclusion. HER2-specific T cells obtained by the protocol we developed characterized by a high level of cytotoxicity against HER2-expressing tumor cells and which are mostly represented by T memory cells, especially TSCM subset. Obtained T cells could be used for eliminating tumor cells and prevent tumor relapse after the primary tumor burden deletion in HER2-overexpressed cancer patients.

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**P.B4.03.16**

The newly-arisen Devil Facial Tumour disease 2 (DFT2) reveals gradual loss of MHC class I as a mechanism for the emergence of a contagious cancer

A. Caldwell, R. Coyley, M. R. Stammnitz, T. C. Brumme; University of Cambridge, Cambridge, United Kingdom; 2University of Cambridge, Cambridge, United Kingdom; 3University of Southern Denmark, Odense, Denmark; 4University of Tasmania, Hobart, Australia.

Devil Facial Tumour Disease 2 (DFT2) is a recently discovered contagious cancer circulating in the Tasmanian devil (Sarcophilus harrisii), a species which already harbours a more widespread contagious cancer, Devil Facial Tumour 1 (DFT1). Here we show that in contrast to DFT1, DFT2 cells express major histocompatibility complex (MHC) class I molecules, demonstrating that loss of MHC is not necessary for the emergence of a contagious cancer. However, the most highly expressed MHC class I alleles in DFT2 cells are non-polymorphic or common among host devils, reducing immunogenicity in a population sharing these alleles. In parallel, MHC class I loss is emerging in vivo, thus DFT2 is mimicking the evolutionary trajectory of DFT1. Based on these results we propose that contagious cancers can exploit partial histocompatibility between the tumour and host, but selective pressure from the immune system will drive loss of allelic antigens, which could facilitate widespread transmission of DFT2. Funding: Leverhulme Trust project grant RPG-2015-103.

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**P.B4.03.17**

TACS - traceless affinity cell selection


Traceless affinity cell selection is a novel approach for positive cell isolation, which completely avoids the use of high affinity antibodies that often lead to irreversible cell binding and cause undesired stimulation of target cells. In contrast to fluorescence-activated cell sorting (FACS) and magnetic-activated cell-sorting (MACS), traceless affinity cell selection utilizes a principle, which we have termed “tag-activated cell sorting” or TACS. TACS comprises an innovative immune-affinity chromatography, whereby target cells are captured by covalently immobilized and that of the column matrix via an epitope tag. Specifically, low affinity Fab-fragments harboring a C-terminal Streptag® (Fab-Streps) are attached to Strept-Tactin®-coated cell grade agarose, and this miniaturization of Fab-Streps promotes their binding to target cells with high avidity. Hence, when a single-cell suspension is passed through a Fab-Strep/Strept-Tactin® column, target cells adhere to the affinity matrix based on the exclusive binding of the Fab-Strep to the target cell. Non-target cells are washed away efficiently. Finally, the addition of biotin triggers the elution of the target cells, and the Fab-Streps spontaneously self-dissociate from the cell surface due to the reduced affinity. This novel “Fab-TACS®” procedure delivers label-free, non-activated target cells of highly reproducible quality and purity directly from a variety of single cell suspensions, e.g. whole blood,uffy coat or rodent spleen, allowing a multitude of downstream applications. Manual Fab-TACS® gravity columns as well as fully automated Fab-TACS® cell isolation using the FABian® bench top instrument provide convenient and cost efficient options for conducting standardized TACS experiments in the laboratory.

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**P.B4.03.18**

Effect of long-term IFN exposure on CD8+ T cell functionality

J. Mikulec, M. Hofmann*, R. Thimme*, R. Bartenschläger*; 1Division of Virus-associated Carcinogenesis, German Cancer Research Center, Heidelberg, Germany; 2Department of Medicine II, University Hospital Freiburg, Freiburg, Germany, 3Department of Infectious Diseases, Molecular Virology, University Hospital Heidelberg, Heidelberg, Germany.

Chronic infection with the hepatitis C virus (HCV) is a major risk factor for serious liver diseases and affects around 1% of the population. A hallmark of HCV is the high propensity to establish persistence, which occurs in around 80% of infected individuals. This is achieved by suppression of both innate and adaptive immune responses. Innate immunity, most notably induction of interferons (IFNs), is the first line defense limiting viral replication and determining the outcome of an infection. However, it has been shown that sustained activation of the IFN system in case of persistent lymphocytic choriomeningitis virus infection has detrimental impact on T cell response. Elevated IFN signature is observed in chronically HCV-infected patients; cells specific for HCV-specific T cell responses are profoundly downregulated and that persistent activation of the IFN system might dampen T cell response also in chronic hepatitis C. To address this hypothesis we investigated the IFN system in case of persistent HCV-specific CD8+ T cell function. For this purpose, we take advantage of a cell culture model, which is based on a HCV replication-containing cell line stably transduced with the HLA-A2 gene. These cells are used as target cells to measure biological activity of HCV-specific CD8+ T cells that have been cultured in the presence of HLA-A2 for extended time spans. Furthermore, we will characterize the IFN-induced changes of CD8+ T cells, including their differentiation status, the expression of inhibitory molecules and all transcription factors. By using these approaches we want to determine to which degree long-term IFN-treated T cells alter their responsiveness to HCV-infected target cells.

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**P.O4.03.14**

**P.O4.03.15**

**P.O4.03.16**

**P.O4.03.17**

**P.O4.03.18**

Abstracts of the 5th European Congress of Immunology - EC1 2018 - Amsterdam, The Netherlands
POSTER PRESENTATIONS

P.B4.03.19

Analysis of genes involved in antigen presentation to MR1T cells
A. Vaschoni, J. Spagnuolo, L. Mori, G. De Liberis; University Hospital and University of Basel, Basel, Switzerland.

The MHC class I-related (MR1) protein is a non-polymorphic molecule presenting bacterial metabolites to MR1-restricted MAIT cells. We have identified a novel population of MR1-restricted T cells that recognize unidentified antigenic molecules belonging to diverse classes and with small-molecular-weight. MR1T cells have polyclonal TCRs and display heterogeneous antigen specificities, recognize and kill different tumor cells in MR1-restricted manner. Activated MR1T cells release various cytokines supporting different transcription factors. Molecular mechanisms underlying the generation of self antigens presented by MR1, their loading onto MR1 and presentation to T cells remain unknown. To identify candidate genes involved in such functions, we transduced MR1-overexpressing A375 melanoma cells with a CRISPR knock-out library (containing 123 411 sgRNAs encompassing the whole human genome) and killed them with MR1T cells, enriching for surviving A375 cells. Guide RNAs amplified from the genomes of surviving A375 cells were deep sequenced and analysed using the edgeR package. Heatmap shown based on the strict standardized mean difference (SSMD) calculated for the two most enriched/depleted guides for each gene target, considering an SSMD ±3 for enriched and ±5 for depleted guides, compared to unknilled cells. Gene ontology analysis of significantly enriched or depleted sgRNAs revealed genes involved in different cell functions, the most abundant being those with a role in primary metabolism. Other identified genes contribute to cell adhesion, protein trafficking, metabolic transport, and RNA stability. We are currently validating selected genes that might participate in the generation/degradation of MR1-stimulating antigens and in MR1-restricted antigen presentation.

P.B4.03.20

Functional characterization of gd T cells in human colon tumours
W. Rodin1, P. Sundström1, F. Ahlmanner1, E. B. Lindskog1, M. Quinding-Järbrink1; 1Dept. of Microbiology and Immunology, Göteborg, Sweden, 2Dept. of Surgery, Göteborg, Sweden.

In colorectal cancer, tumour progression and patient outcome is affected by cytokine balance in the tumours. IFNγ, TNFα and Granulocyte B production are all associated with favorable patient outcome, while high IL-17 expression is associated with accelerated tumour progression. However, the knowledge of regulation and activation of intraepithelial T cells in colon cancer is still limited. The aim of this study was to characterize intestinal intraepithelial gd T cells in colon tumours and unaffected tissue as well as to determine their capacity to produce cytokines affecting tumour progression, using flow cytometry. We show that the frequencies of Vδ1+ gd T cells and CD8+ T cells are reduced in the epithelium of colon tumours compared to unaffected tissue. Intraepithelial T cells in both tissues express markers associated with an activated memory phenotype and moderate levels of PD-1. On a per cell basis the tumour epithelium contains higher frequencies of y6 and aδ T cells producing IFNγ and TNFα compared to the unaffected tissue. Likewise, frequencies of IL-17 producing cells are also higher in IELs from tumours, but much lower than the T1– associated cytokines. Granulocyte B production was observed in y6 and aδ T cells both before and after activation and is also activated at higher levels in tumour IELs compared to IELs from unaffected tissue. Taken together, this study indicates that IELs actively contribute to the cytokine balance in colon tumours with a T1– dominated profile, which may influence tumour progression and patient outcome.

P.B4.03.21

Depletion of intraepithelial regulatory T cells by the anti-ICOS antibody KY1044 in combination with immune checkpoint blockade enhances the anti-tumour response in pre-clinical models

Inducible T cell costimulator (ICOS/COD278) is a CD28 superfamily member that is induced when T cells are activated. Although ICOS expression levels vary in different T cell subtypes and in different tissues, in preclinical mouse tumour models, we have observed that nearly all T regulatory cells (TRegs; CD4+/FOXP3+) express ICOS on their surface and that the expression level of ICOS on TRegs is significantly higher than that on effector T cells (TEffs). In addition, ICOS expression on TRegs in the tumour microenvironment is higher than that of TRegs in the blood or spleen, which makes it a strong candidate for cancer immunotherapy via TReg depletion. By immunizing Kymeic™, in which the endogenous icos gene has been knocked out, we have identified a novel, cross-reactive, fully human antibody called KY1044 that selectively binds to dimeric ICOS (Fc fusion protein) with an affinity of less than 2 nM. Using in vitro and in vivo approaches we demonstrate that KY1044 has dual mechanisms of action: (1) it promotes the preferential depletion of intraepithelial ICOS+/- TRegs resulting in an increase in the TEff:TReg ratio in the TME; and (2) it co-stimulates ICOS+/- TEff cells. Notably, our pharmacodynamic studies demonstrated long-term durable depletion of TRegs and a significant increase in response to KY1044 treatment, in an effect that is crucially dependent on CD8+ but not CD4+ T cells. In summary, our data demonstrate that targeting ICOS with KY1044 is a valid approach for modulating the immune system and for inducing a strong anti-tumour response in multiple indications.

P.C1.01.01 Maintenance and local regulation of tissue specific immunity - Part 1

P.C1.01.01 Negative Checkpoint Receptor (NCR) expression in Graves’ disease thyroid tissue
D. Álvarez-Serrà1, A. Marín-Sánchez1, P. Ruiz-Blázquez2, C. Iglesias-Felpú3, R. Nucifora4, O. González-López5, A. Casteras5, G. Obid6, P. Pujol-Barreil7; 1Vall d’Hebron Research Institute, Barcelona, Spain, 2Department of Immunology, Hospital Universitari Vall d’Hebron, Barcelona, Spain, 3Department of Pathology, Hospital Universitari Vall d’Hebron, Barcelona, Spain, 4Vall d’Hebron Institute of Oncology, Barcelona, Spain, 5Department of Surgery, Hospital Universitari Vall d’Hebron, Barcelona, Spain, 6Department of Endocrinology, Hospital Universitari Vall d’Hebron, Barcelona, Spain.

It has been reported that autoimmune thyroiditis occurs frequently after cancer immunotherapy with anti-PD-1/PD-L1. Since PD-L1 can be induced by IFNγ and in Graves’ Disease (GD) thyroid there is a clear IFNγ signature, we predicted that thyrocytes, even in focal thyroiditis, would express PD-L1. Anti-PD-L1 treatment would then exacerbate focal thyroiditis and lead to clinical thyroid autoimmunity. The results here presented suggest another scenario. Cryostat sections of GD, multinodular goitre and control lymphoid organs were stained by multicolour immunofluorescence for PD-1, PD-L1, as well as for HLAI and HLAII, Cytokertatin-18, TPO and phenotypic markers. Against our prediction, in most GD glands, PD-L1 was undetectable in thyrocytes or present at very low level, certainly much less conspicuous than HLAII. Importantly, PD-L1 was highly expressed by the infiltrating T cells. Primary thyroid and thyroid-derived cell lines cultures were supplemented with increasing doses of IFNγ and stained at 24h-48h to assess HLA-I/II, PD-L1 and PD-L2 expression induction. PD-L1 and PD-L2 expression was readily induced by IFNγmaga in thyrocytes cultures in a time and dose dependent manner, as assessed by flow cytometry and qPCR, demonstrating the capability of thyrocytes to express PD-L1 ligands. Overall these results suggest that the autoimmune thyroiditis linked to anti-PD-1/PD-L1 drugs blocking the interaction between PD-1 positive T cells and thyrocytes expressing PD-L1 or PD-L2. The factor(s) inhibiting thyrocytes PDLL induction in vivo remain to be elucidated.

P.C1.01.02 Sexual dimorphism in mechanisms controlling development of CD4+ T cell response in collagen-induced arthritis
B. Bujanov1, N. Arsenović-Ronić1, M. Dimitrijević2, N. Maksic-Aleksić1, D. Kosce1, P. Filipović1, M. Stojanović1, G. Leposavić1; 1Department of Microbiology and Immunology, University of Belgrade Faculty of Pharmacy, Belgrade, Serbia, 2Department of Immunology, Institute for Biological Research “Siniša Stanković”, University of Belgrade, Belgrade, Serbia, 3Department of Physiology, University of Belgrade Faculty of Pharmacy, Belgrade, Serbia, 4Immunology Research Centre “Branislav Janković”, Institute of Virology, Vaccines and sera “Tolikon”, Belgrade, Serbia.

Introduction: Considering sex bias in rheumatoid arthritis prevalence, influence of biological sex on the disease development in Dark Agouti rat collagen II-induced arthritis (CIA) model of the human disease was examined. Methods: Sex bias in CD4+ T cell responses in in vivo model of the disease site of immunization in preclinical CIA and polyclonal (daily) inflamed joints at the peak of CIA) lymph nodes (LNs) and mechanisms controlling their development were examined using flow cytometry and/or ELISA/qRT-PCR. Results: In both inguinal and popliteal LNs greater number of CD4+CD25+Foxp3+ cells, presumably activated effector T cells, was found in females compared with males, and they exhibited greater CIA-specific proliferation. Consistently, more IL-17+, IFNγ+ and IL-17+IFNγ+ T cells were retrieved from both inguinal and popliteal rat LNs. Moreover, more GM-CSF+ and IL-17+IFNγ+ GM-CSF+ T cells were retrieved from female compared with male rat popliteal LNs. On the other hand, lower frequency of PD-1+ cells among D4+CD25+Foxp3+ regulatory T cells (Tregs) from popliteal and inguinal LNs suggested lower suppressive capacity of their Tregs. Additionally, from female rat popliteal LNs fewer Tregs were recovered. Furthermore, the number of regulatory LN B cells was lower in females. Moreover, compared with males, in females was shifted LN INFγ+IL-4+ T-cell ratio towards the former, and accordingly serum CIA-specific IgG2a/IgG1 antibody ratio was shifted towards pathogenic IgG2a antibodies. Conclusion: The study suggests that a less efficient control of (auto)immune Th1/Th17 cell responses during CIA development contributes to sex bias in the susceptibility to CIA. (Grant 175050, MESTO of Serbia)
POSTER PRESENTATIONS

P.C1.01.03
ΔdblGATA mice are resistant to experimental autoimmune encephalomyelitis
B. Ciric, C. Hawang, A. Rostami;
Thomas Jefferson University, Philadelphia, United States.

GATA-binding factor 1 (GATA-1) is a transcription factor expressed in certain hematopoietic cells, governing their development and function. GATA-1 is expressed in myeloid cells but not in lymphoid cells, such as T cells. Since knockout of GATA-1 is embryonic lethal, mouse lines lacking individual enhancers to the GATA-1 gene have been generated. Lack of a particular enhancer selectively reduces, or precludes GATA-1 expression in some cell types, while in others its expression remains normal. We use mice lacking ΔdblGATA enhancer (ΔdblGATA mice), which are devoid of eosinophils. We found that ΔdblGATA mice are resistant to both direct and adoptive EAE, ΔdblGATA mice develop weaker myelin-specific Th responses upon immunization for EAE induction, indicating a defect in priming immune responses. The resistance of ΔdblGATA mice to adoptive EAE indicates a deficiency in effector mechanisms of CNS inflammation. We have ruled out lack of eosinophils as the reason for EAE resistance of ΔdblGATA mice by demonstrating that another mouse strain (PHIL), which also lacks eosinophils, develops normal EAE. These findings show that ΔdblGATA mice have defects in both the priming and effector phases of disease. Additionally, in vivo and in vitro ΔdblGATA mice had fewer inflammatory monocytes and myeloid dendritic cells in blood, draining lymph nodes and the CNS. Our findings thus far suggest that an unknown defect in monocytes/dendritic cells causes resistance to EAE in ΔdblGATA mice.

P.C1.01.04
MAF expression in regulatory and conventional T cells controls gut homeostasis
C. Imbrotta1, M. Leblond2, D. Velin3, H. Bouchoureurre4, D. Speiser5, G. Verdier6;
1Clinical Tumor Biology & Immunotherapy Group, Department of Fundamental Oncology, University of Lausanne, Epalinges, Switzerland, 2Department of Gastroenterology and Hepatology, CHUV, Lausanne, Switzerland, 3Service of Gastroenterology and Hepatology, CHUV, Lausanne, Switzerland, 4Unilabs, Epalinges, Switzerland, 5Clinical Tumor Biology & Immunotherapy Group, Department of Fundamental Oncology, University of Lausanne, CHUV, Epalinges, Switzerland.

The maintenance of homeostasis in the gut is a major challenge for the immune system. In an attempt to characterize the role of the transcription factor MAF in T cells, we demonstrated that it is a key regulator of homeostasis in the gut. Mouse knockout for maf in all T cells developed a spontaneous late-onset colitis, correlating with the disappearance of FOXP3+ Treg+ T cells from the colon that normally express high level of MAF and is the major source of IL-10. We demonstrated that classical T regulatory cells inactivated for maf are defective in controlling colitis development in a co-transfer model and are not able to cope for the defect of RORγt+ Treg in the colon. Furthermore we showed that Treg-specific maf KO mice are not sufficient to induce colitis and that the deletion of maf in conventional T cells is also necessary for the development of the disease.

P.C1.01.05
TCR repertoire of PD-1+ and CD137+ T-cells from synovial fluid of patients with spondyloarthropathies
E. A. Komech1, A. D. Kaltkova1, T. V. Kortova1, E. Y. Loginova1, E. I. Shmidt1, Y. B. Lebedev2, I. V. Zyvagin2,1;
1Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation, 2Pirogov Russian National Research Medical University, Moscow, Russian Federation.

To investigate whether IL-37 can alleviate PBC, we used adeno-associated virus as a vector to express IL-37 in the liver of PBC mice. We found that the features of PBC, including activation of liver cells, infiltration of PMNs in liver, and activation of PMNs in liver were reduced in AAV-hIL-37 injected PBC mice. However, polymorphic nuclear cells (PMNs) in liver were increased in AAV-hIL-37 injected PBC mice. To further extend whether IL-37 recruit PMN in the immune system, we focused on TCR repertoire of activated PD-1+ and CD137+ T-cells from synovial fluid (SF) samples of patients with ankylosing spondylitis (AS) and psoriatic arthritis. Using quantitative molecular-barcoded 5'-RACE, we performed TCRβ repertoire profiling of activated T-cells: CD3+PD-1+SF, CD3+CD4+SF, and CD3+CD8+SF, that were FACs-sorted from SF samples of patients. From 57 to 83.7% of T-cells from SF were PD-1+ and CD137+. T-cells from PD-1+ and CD137+ T-cells were found to be different from SF-1- cells. Most of CD8+CD137+ T-cells were found in PD-1+ subset of the same donor. Only a few clonotypes of activated fractions were shared between donors, including one clonotype from recently identified AS-associated TCRβ motif. In repertoire of the activated subsets we observed several low abundant clonotypes with known specificities for CMV, EBV, Influenza and melanoma proteins. Our results show that the majority T-cells from synovial fluid of patients with spondyloarthropathies are activated. T-cells clonotypes of activated fractions are rather unique for each donor. Presence of clonotypes matching AS-associated TCRβ motif in activated T-cells subsets of patients with spondyloarthropathies supports their role in pathogenesis of the disease.

P.C1.01.06
IL-37 increases inflammation in murine immune-mediated liver diseases
C. Lin, Y. Chuang;
Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, Taipei, Taiwan.

IL-37 is an emerging 1 family cytokine with anti-inflammatory effects on innate and adaptive immunity that shows benefit on several autoimmune diseases. Primary biliary cholangitis (PBC) is a chronic autoimmune disease that occurred in the liver with innate adaptive immune cell infiltration in the portal triad, followed by fibrosis and cirrhosis. To investigate whether IL-37 can alleviate PBC, we used adeno-associated virus as a vector to express IL-37 in the liver of PBC mice. We found that the features of PBC, including hepatic lymphoid infiltrations, serum autoantibody expression and hepatic histopathological changes were improved in AAV-IL-37 infected PBC mice. To further extend whether IL-37 recruit PMN in the immune mediated liver disease, we used Concanaavalin A (Con A) induced hepatitis mouse model. Compared to AAV-mock group, Con A injected mice administered with AAV-IL-37 had significantly increased in not only PMNs but also NK, NKT, and dendritic cells in the liver. In addition, serum IFN-γ and TNFα increased in AAV-IL-37 infected Con A mice. In conclusion, IL-37 induces PMN infiltration in autoimmune cholangitis and Con A induced hepatitis and shows pro-inflammatory effects in Con A induced hepatitits.

P.C1.01.07
Deletion of the mineralocorticoid receptor in myeloid cells leads to reduced EAE severity
F. Lüder1, E. Montes-Cobo2, N. Schweingruber1, A. Flügel1, H. Reichardt1;
1Institute of Multiple Sclerosis Research, Göttingen, Germany, 2Institute of Cellular and Molecular Immunology, Göttingen, Germany.

Myeloid cells play important roles in different crucial stages during the pathogenesis of multiple sclerosis and its animal model experimental autoimmune encephalomyelitis (EAE). Peripheral myeloid cells act as APCs for the initial activation of autoreactive T cells in peripheral lymphoid organs, whereas local myeloid cells are presumably responsible for their re-activation within the CNS. Myeloid cells such as monocytes and macrophages, but also local CNS-resident ones such as microglia, can differentiate towards distinct phenotypes, M1 myeloid cells being more related towards inflammation and autoimmunity and M2 myeloid cells being more related to tissue repair and anti-inflammatory processes. We generated a mouse model specifically lacking the mineralocorticoid receptor (MR) in myeloid cells (MR-ko mice). In these mice, the phenotype of bone marrow-derived and peritoneal macrophages was modulated towards M2 including differential phagocytic and migratory properties and NO metabolism. Furthermore, these mice presented with diminished clinical symptoms and ameliorated histological hallmarks of neuroinflammation after EAE induction by MOGd35-55 and CFA. T cells in peripheral lymphoid organs of these mice produced less pro-inflammatory cytokines while their proliferation and the abundance of regulatory T cells were unaltered. In the CNS, the numbers of inflammatory monocytes and reactive microglia in MR-ko mice were significantly lower and they also adopted an M2-polarized phenotype based on their gene expression profile, presumably explaining the ameliorated neuroinflammation. Thus, the lack of the MR in myeloid cells impacts CNS autoimmunity by either direct effects and/or modulation of the adaptive immune system.

P.C1.01.08
Tissue-specific immune dysregulation in human autoimmune thyroiditis
A. Mohr1, C. Trésilet1, N. Monot1, A. Basvois1, D. Albiere1, L. Leenhardt1, G. Gorachov1, M. Miyora1;

Introduction: Peripheral abnormalities in Th17 and regulatory T cells have been observed in autoimmune thyroid diseases (AITDs) that include Hashimoto’s thyroiditis (HT) and Grave’s disease (GD). However, it is still unclear whether such abnormalities occur in the thyroid tissue. Materials and Methods: PBMCs and thyroid tissue infiltrating immune cells from 18 patients with HT, 10 patients with GD and 30 control donors (CD) have been analyzed by flow cytometry in order to study T cells and ILC subsets.
RESULTS: We did not observe the previously reported increase of peripheral TH17 cells or the decrease of circulating Treg cells in HT and GD patients. CCR5-PD1+ T cells and IL-17+ ILC3 were significantly increased among immune cells infiltrating thyroid from patients with both HT and GD. No modification in FOXP3 effector Treg (Fr II) cells was observed in AIIDTs tissue when compared to controls, while IL-2 producing FOXP3+CD4+ T (Fr III) cells were increased. In addition, the presence of FOXP3-FOXP3+CD4+ TFR-like cells with low levels of CTLA-4 was also suggestive of defective local immune dysregulation in AIIDTs.

CONCLUSION: AIIDTs are characterized by the occurrence of tissue immune dysregulation as the presence of TH and ILC3, two populations involved in secondary and tertiary lymphoid organ development, and IL-2 producing FOXP3+CD4+ TFR-like predominating in patients. Those abnormalities may promote the local production of autoantibodies.

P.C1.01.09

Immunological biomarkers of autoimmune liver disease in patients without clinical symptomatology

A. Novasi1, M. López1, J. Molino2, A. Jaradov1;
1Maimonides Biomedical Research Institute of Cordoba (IMIBIC)/ Reina Sofia University Hospital/ University of Cordoba, Cordoba, Spain, 2Department of Clinical Analyses, Reina Sofia University Hospital, Cordoba, Spain, 3Department of Allergy and Immunology, Reina Sofia University Hospital, Cordoba, Spain.

Primary biliary cirrhosis (PBC) is an autoimmune disease which results in a slow and progressive destruction of the liver small bile-ducts. PBC is more common in medium-aged women and its diagnosis is usually hazard-made according to clinical symptoms, such as tiredness, itching and an increased level of liver-enzymes routinely examined. Autoantibodies against gp-210 and sp-100 are found in association with PBC, together with anti-centromarial antibodies. The aim of this study was to analyze demographics and clinical characteristics of serum samples screened for autoantibodies with positivity to anti-gp-210 and sp-100 antibodies.

In this study 75 patients were included. Serum sp-100 and/or gp-210 autoantibune biomarkers were detected by immune-blot according to the immunofluorescence result of autoantibodies on Hep-2 cells. Levels of transaminases were measured by chemiluminescence. The diagnostic suspicion was registered.

The 92% of patients were women. Medium age of all patients was 59±13.5. Anti-sp-100 and anti-gp-210 were positive in 49 (65.3%) and 20 (26.7%) patients, respectively. In 5 patients (6.7%) both antibodies were detected. Medium level of liver-enzymes was 43.9±42.3 for aspartate-amiontransferase, 43.07±50.1 for alanine-amiontransferase, 171.1±246.9 for gamma-glutamyl-transferase and 153.9±180.2 for alkaline-phosphatase. The majority of the samples came from the Rheumatology Department (29.3%), followed by those from the Digestive Department (21.3%). Only 17 (22.7%) samples belonged to patients with the clinical suspicion of PBC.

Samples from medium age women routinely examined with an increased level of transaminases may exhibit a positivity to PBC autoantibody biomarkers. Interestingly, the majority of them are not clinically suspicious of suffering it.

P.C1.01.10

Assessment of the DFS-70 (anti-dense fine speckled 70 antibodies) specificity by CIA (Chemiluminescent immunoassay) and blot in 96 serum samples with the ICAP AC-2 autoimmune rheumatic diseases pattern

D. Monzón Casadei1, M. De Juan1, M. Imaz Ocharan1, M. Rey Rey1, L. Aragón Irisquii2, Á. Prada Iñurrategui3;
1Osakidetza, San Sebastián, Spain, 2Osakidetza, Bilbao, Spain.

INTRODUCTION: The nuclear dense fine speckled (ICAP AC-2) pattern in Hep-2 are observed in autoimmunity laboratories. These autoantibodies have been detected with low frequency in systemic autoimmune rheumatic diseases (SARD), in healthy individuals and in patients with other diseases.

OBJECTIVE: We selected serum from 96 patients based on an immunofluorescence screening of ANA ( Tittle ≥ 1/160 and ICAP AC-2 pattern). We investigated anti DFS-70 antibodies using chemiluminescence (CIA) immunoassay and blotting..

MATERIAL AND METHODS: We analyzed 96 sera by IFA screening (Euroimmun). DFS-70: CIA (Quanta Flash Werfen) and Blot (Euroimmun). ANAs specificities: Blot (Euroimmun). dsDNA antibodies: Enzyme immunoassay (Thermofisher) and IFA (Cinthidio lucilize).

RESULTS: 1: DFS antibodies. From the 96 ICAP-AC2: 12/96 were positive by blot and 6/96 by CIA. Using a binomial distribution with an uninformative beta prior, we estimated that the probability the Blot detects more positive results than the CIA is 0.9305.

2: ANAs specificity

Three patients DFS-70 positive by blot were positive for RNP, Histones and Cenp-B. An ICAP AC-2 positive DFS-70 negative cohort (n=14) were positive for histones (2p) nucleosomes (1p), Scl-70 (1p70), Sp-100 (4p), and others.

The ICAP AC-2 positive cohort determined by IFA (n=79) resulted negative for anti DFS-70 and other nuclear specificities.

3: Anti-dsDNA.

All the patients were negative. .

CONCLUSIONS: 1: DFS-70 monospecific antibodies are present in a low fraction of ICAP-AC2.

2: The Blot has more positive results than the CIA.

3: Besides DFS-70, other unknown specificities might show IFA AC-2 pattern.

P.C1.01.11

The role of the tetraspanin CD82 in rheumatoid arthritis synovial fibroblast migration and inflammation

E. Neumann1, M. Schwarz2, M. Hülser3;
1Dept Rheumatology and Clinical Immunology, Campus Kerckhoff, Justus-Liebig-University Giessen, Bad Naueheim, Germany, 2Divis. Vascular Surgery, Kerckhoff Klinik GmbH, Bad Naueheim, Germany, 3Dept Plastic, Hand and Reconstructive Surgery, BGU, Frankfurt, Germany, 4Dept of Orthopaedics and Trauma Surgery, Agapeioz Markus Hospital, Frankfurt, Germany.

INTRODUCTION: Tetraspanins are membrane adaptors altering cell-cell fusion, receptor-mediated signal transduction and cell motility via interaction with membrane proteins including adhesion molecules such as integrins. CD82 is expressed in several malignant cells and a well described tumor metastasis suppressor. Rheumatoid arthrits (RA) is based on synovial inflammation and joint destruction driven in part by activated synovial fibroblasts (SF) with an increased migratory potential. CD82 is upregulated in RA SF and osteoarthritis (OA) SF and within RA vs. OA synovial tissue. Therefore, the role of CD82 in RA SF was evaluated.

METHODS: Double-immunofluorescence for CD82 and integrins, proinflammatory stimuli induction of CD82, lentiviral CD82-overexpression, siRNA-mediated knockdown, RASF migration and attachment assays towards plastic/matrigel, RASF-binding assays to endothelial cells (EC), and evaluation of CD82 expression during long-term invasion in the SCID-mouse model were performed.

RESULTS: CD82 was significantly induced by proinflammatory stimuli (e.g. RA SF. TNFa 9.55-fold; IL-12 15.42-fold). In RA-synovium, CD82 was expressed in RA SF close to vessels, lining layer, sites of cartilage invasion, and colocalized with integrins involved in tumor metastasis suppression and RASF attachment. CD82 overexpression reduced RA SF migration (CD82 174±50,70, NTP 74.5±70,1, p=0.044) and matrix adhesion (147.7% vs. NTP p=0.0166) whereas RA SF-EC interaction was reduced (60.7% vs. NTP p=0.0015). In SCID mice, the presence of CD82 on cartilage-invading RA SF was confirmed.

CONCLUSION: CD82 contributes to RA SF migration to sites of inflammation and tissue damage but also to perpetuation of RA SF to articular matrix.

P.C1.01.12

Association of HLA-A alleles with vitiligo in Turkish people

Y. Hayran, G. O. Ergen, F. Ozman;
Department of Basic Oncology, Cancer Institute, Hacettepe University, Ankara, Turkey.

Background and Aim: Vitiligo is an autoimmune disease of the skin that results in the destruction of melanocytes and the clinical appearance of white spots. The aim of this study was to investigate the association of HLA-A alleles with vitiligo in patients with vitiligo. Patients and Methods: The study included 71 patients with vitiligo and the control group included 100 unrelated healthy donors. HLA-A typing was performed by using PCR-SSO method at low resolution level. Results: 71 (40%) patients with Vitiligo included in our study which consist of 46 (64.8%) vitiligo vulgaris. Extensiveness of disease was 77.5% (1.45%) and commence age (mean) of the disease was 26.5 years. 32.5% (11) male patients had positive family history. HLA-A*02 allele frequency was found to be significantly higher in vitiligo patients than control group (p<0.023). Each allele increase the risk of vitiligo by two fold (OR=2.19 3.34). HLA-A*02 allele frequency was similar between the sub-types of vitiligo. HLA-A*02 allele frequency was also found to significantly correlated with commence age of the disease (p=0.026 10.266). When compared to the patient, HLA-A*11 was found to significantly higher in controls (p=0.008). HLA-A*11 was found to be protective for vitiligo disease and each allele was preventive as 3.34 fold (p=0.0118). There was no statistical correlation between HLA-A*02 allele frequency and extensiveness of the disease (VASI), degree of pigmentation, disease activity(VADI), associated auto-immune disease and the presence of vitiligo in the family. Conclusion: This results suggest that HLA-A*02 allele might be positively associated with vitiligo in Turkish people.
POSTER PRESENTATIONS

**P.C1.01.13**
**Mechanisms impaired in the pathogenesis of multiple sclerosis**

L. Panagoulias, 1, A. Georgakopoulou, 2, A. Kostadinova, 1, V. Kostadinova, 1, J. Prechl, 3, E. Kourepini, 3, E. Pappou, 3, A. I. Tchorbanov, 1, I. Panagoulias, 1

1Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 2Institute of Immunology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 3Biomedical Research Foundation of the Academy of Athens, Athens, Greece.

In MS, pathogenic Th cells (mainly Th1 and Th17) recognize myelin antigens and contribute to the damage to the CNS. An important unresolved issue of MS pathogenesis is at which stage of Th-cell differentiation errors occur, at the molecular level, that result in the development of autoreactive Th-cells. We previously showed that in healthy individuals the IL-2 gene is repressed in naive Th-cells by the transcription factor Ets-2, that binds to ARRE-2 element (distal NFAT binding site) of the proximal IL-2 promoter, pointing to Ets-2 as a crucial factor in the development of early events of Th-cell differentiation. Importantly, we also demonstrated that Ets-2 suppresses the expression of other genes with Ets-2 binding sites, including cytokines and HIV-1. Our results from 10 patients with remitting-relapsing MS (4M/6F, age: 24-38y, disease duration: 2.7-16y; EDSS: 1.2-5), and 10 age/matched healthy controls, show significantly reduced mRNA and protein synthesis of Ets-2 in naive Th-cells from MS patients, lack of Ets-2 binding to the ARRE-2 of the IL-2 promoter in vitro and in vivo, and significantly higher constitutive expression levels of cytokines in the patients’ Th-cells (IL-2, IL-17 in naive Th-cells and TNF-α, IFN-γ in memory Th-cells). We also found significantly higher Ets-2 expression in undifferentiated murine Th-cells compared to in vitro differentiated Th-cell populations, and elevated Ets-2 expression in Th-cells from mice resistant to EAE. We suggest that in MS patients low-level synthesis and dysfunction of Ets-2 in Th-cells are responsible for downstream aberrant Th-cell differentiation resulting in the creation of pathological Th1 and Th17 cells.

**P.C1.01.14**
**Dominant protection from HLA-linked autoimmune disease by antigen specific regulatory T cells**


1Medical School, University of Patras, Patras, Greece, 2Medical School, University of Melbourne, Melbourne, Australia, 3Biomedical Research Foundation of the Academy of Athens, Athens, Greece.

Susceptibility and protection against autoimmune diseases including Rheumatoid Arthritis, Multiple Sclerosis, Type I Diabetes and Goodpasture disease (GPD) is associated with particular human leukocyte antigen (HLA) alleles. T cell responses against HLA self-epitopes are considered key drivers of autoimmunity, however the molecular mechanisms that link specific HLA alleles to the initiation or suppression of autoimmunity are presently unclear. Typically, the diversity of T cell self-epitopes and the breadth of HLA associations preclude a definitive analysis of the underlying molecular mechanisms. GPD is an HLA-linked autoimmune renal disease, where the immune response is dominated by a single CD4+ T cell self-epitope (a3) derived from type IV collagen. While HLA-DR15 confers a markedly increased disease risk, the HLA-DR1 allele is dominantly protective. HLA-DR15 and HLA-DR1 exhibit distinct peptide repertoires and binding preferences, and the a3 epitope is presented in different binding registers. The difference in a3 presentation is associated with functionally distinct T cell repertoires: HLA-DR15/a3 tetramer+ T cells in HLA-DR15 transgenic mice exhibit a conventional T-cell phenotype [Tconv] that secretes pro-inflammatory cytokines. In contrast, HLA-DR1-a3 tetramer+ T cells in HLA-DR1 and HLA-DR15/DR1 transgenic mice are predominantly CD4+Foxp3+ regulatory T cells (Treg cells) that express regulatory cytokines. HLA-DR15-induced Treg cells confer resistance to HLA-DR15 transgenic mice to the rechallenge for the dominantly protective effect of HLA in autoimmune disease, whereby HLA polymorphism shapes the relative abundance of self-epitope specific Treg cells that leads to protection or causation of autoimmunity.

**P.C1.01.15**
**Staphylococcus aureus induced Th17 cell accumulation aggravates renal autoimmune disease**

D. Reimers, 1, P. Bartsch, 1, U. Panzer, 1, S. Klinge, 1, H. Rohde, 1, C. Krebs, 1, H. Mittrücker;

1Institute of Immunology - University Medical Center Hamburg-Eppendorf, Hamburg, Germany, 2Ill. Medical Clinic and Polyclinic - University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

CD4+Th17 cells are defined by their production of IL-17 cytokines and the expression of the transcription factor RORγt. Th17 cells are responsible for the clearance of extracellular pathogens. However, these cells also participate in autoimmune diseases such as crescentic glomerulonephritis (cGN). In our project we infect mice with Staphylococcus aureus and characterize the systemic and renal T cell response to infection. In addition, we induce cGN and determine the impact of prior S. aureus infection on the course and severity of renal disease.

S. aureus infection led to infection of the kidneys accompanied by a massive accumulation of T cells at day 10 post infection. Characterization of renal T cells revealed dominant populations of RORγt-positive IL-17A-secreting Th17 cells and γδ T cells. Both T cell populations were rather stable, even 10 weeks after infection increased numbers of renal Th17 and γδ T cells were still detectable. Furthermore, renal RORγt-positive T cells showed a tissue resident phenotype, defined by CD69 expression. 10 weeks after infection, cells from naive and sheep IgG in vivo treated mice showed the same phenotype. As compared to nephritic IgG-tolerized mice, mice with preceding S. aureus infection exhibited augmented renal injury with severe loss of renal function, enhanced glomerular crescents formation and massive tubulo-interstitial damage. In conclusion, our results demonstrate that renal infection and accumulation of Th17 cells correlates with increased autoimmune disease activity. Currently, we aim to identify the underlying mechanisms.

**P.C1.01.16**
**Systemic lupus and reproduction, lessons from the animal models**

G. Boneva, 1, V. Kostadinova, 1, J. Prechl, 2, N. Mihaylova, 2, S. Delimitreva, 2, A. I. Tchorbanov, 1

1Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 2Inmunology Research Group, Hungarian Academy of Sciences, Budapest, Hungary.

Introduction: Systemic Lupus Erythematosus (SLE) is a polygenic autoimmune disorder involving multiple organs that can influence female fertility. Pristane-induced mouse model of SLE is very suitable to study female fertility compared to the healthy animals with the same background. Materials and Methods: Lupus-like symptoms were induced through intraperitoneal injection of hydrocarbon oil in pristane non-autoimmune Balb/c mice. Flow cytometry was used for the detection of CD25/CD69 activated markers and apoptosis assay. The levels of cytokines, autoantibodies in the sera and the number of autoantibody-producing plasmocytes were quantified by ELISA, ELISPOT and protein array. Results: A single i.p. injection of pristane leads to the development of the typical SLE symptoms such as production of different autoantibodies accompanied by massive glomerular depositions of IgG-containing immune complexes in the kidneys, and proteinuria. After hormonal ovarian stimulation, ovulated oocytes were derived from oviducts. Chromatin, tubulin and actin structures were detected by Hoechst 33258, FITC-labeled alpha-tubulin antibody and rhodamine-labeled phallidin, respectively. The total number of obtained matured oocytes was significantly higher compared to the proportion of eggs reaching metaphase II, which was also lower for Lupus mice compared to control animals. For each oocyte, features were described - spindle morphology, actin cap, chromosomal condensation and alignment. Many specific abnormalities in the lupus group were found. Conclusions: Pristane-induced mouse model of lupus exhibited numerous impairments of the reproductive system which may result due to disease activity, autoantibodies or damage in molecular mechanisms through the process of reproduction.

**P.C1.01.17**
**Enrichment of pioneer CD4+ T cells and up regulation of CXCR1 in Experimental Autoimmune Uveoretinitis**

A. Ward, 1, O. H. Bell, 2, D. A. Copland, 2, A. D. Dick, 1, L. B. Nicholson; 1University of Bristol, Bristol, United Kingdom

Non-infectious uveitis is a T cell mediated intraocular inflammatory disease leading to severe visual impairment. Experimental autoimmune uveitis (EAU) is a preclinical model that shares many features of the human disease and can be induced in rodents by transfer of autologous specific lymphocytes. Combined high resolution clinical imaging and flow cytometric assay, enables us to delineate the ratio of transferred and endogenous cells throughout the disease course. As the sole receptor for CXCR1, CXCR1 expression is associated with the retention and survival of CD4+ T cells in the lung and skin. To interrogate the induction of EAU by adoptive transfer of antigen-specific lymphocytes and the role of CXCR1, cells obtained from immunized C3HCR1+/- reporter mice (Ly5.2) were transferred into naive Ly5.1 recipients. Transferred and endogenous cells rapidly accumulate in the eye within 48 hours. As clinical disease progresses to day 7, 20% of the transferred CD4+ cells were GFP positive, increasing to 50% by day 14. This demonstrates selective enrichment, as the transferred cells comprised an increasing fraction of total retinal cell infiltrate.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
These studies suggest that disease-causing antigen specific cells have a long half-life in ocular tissue and/or recirculate extensively. Within the eye, a subset of CD4 cells have a tissue specific phenotype defined by the expression of CKJCR1 which promotes survival or increases retention of specific cells. Long-lived disease-causing cells are therefore a potential candidate for targeted immune-intervention.

P.C1.01.18 Polymorphisms of the cytokine genes TGFβ1 and TNFα in Polish patients with inflammatory bowel disease E. Zakoszielna1, M. Zagdań, A. Surowiecka-Pastewka1, M. Dunik1, 2
1Morsaski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, 2Central Clinical Hospital of the Ministry of the Interior and Administration, Warsaw, Poland.

Introduction: Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract, consisting two clinical entities: Crohn’s disease and ulcerative colitis. TGF-β and TNF-α are two major immunoregulatory cytokines and their signaling pathways play a critical role in IBD development and progression. The aim of the study was to analyse the TGFα and TGFβ1 gene polymorphisms in Polish patients with IBD and its relationship with the secreted cytokines.

Materials and Methods: A total of 42 patients diagnosed with IBD were examined for the TNFA (-308G/A) and TGFβ1 (+29C>T) SNP. As a control group, 100 healthy individuals from the same geographical region were selected. Cytokine levels were measured by ELISA in serum samples.

Results: A significantly increased level of TNFα was observed in patients carrying AA variant of -308G/A polymorphism compared to control. Genotype-phenotype correlation analysis revealed that AA variant was also associated with the development of perianal fistulas. While TGF-β serum level in IBD patients was significantly higher than controls, no significant difference in TGF-β levels between different genotypes exist. Further analysis showed that patients having AA variant of +29C>T SNP suffer from severe disease condition. Conclusions: Our data suggest that TNFA gene polymorphism participate in determination of IBD susceptibility in Polish patients. The cytokine level is significantly modulated in patients with different genotypes of TNFA gene. Further studies of additional SNPs and increasing the number of individuals should be performed to confirm the role of TNF-α and TGF-β.

P.C1.01.19 Expression of HPGD in regulatory T cells prevents tissue inflammation and metabolic dysfunction L. M. Schmidleitner1, Y. Thabet1, E. Schönfeld1, M. Körner2, Z. Abdulatif1, K. Klee1, T. Sadori1, W. Kreib5, K. Subbaramosah3, M. Schneeweiß4, J. SANDER4, N. Ohkura1, A. Wahab1, T. Sonoda1, S. Förster1, H. Wagner1, S. Sakaguchi1, M. Blüher1, C. Brand1, J. Danner3, N. Ferreira4, L. J. Muylaert4, C. Wickenhauser4, S. C. Bony1, J. L. Schützer4, M. D. Beyer4, 1German Center for Neurodegenerative Diseases, Bonn, Germany, 2Life and Medical Sciences (LIMES) Institute, Bonn, Germany, 3Institute for Experimental Immunology, Bonn, Germany, 4Robinson Research Institute, Adelaide, Australia, 5Weill Cornell Medical College, New York, United States, 6WPI Immunology Frontier Research Center, Osaka, Japan, 7University Hospital Bonn, Bonn, Germany, 8Institute of Infectious Immunology, Hannover, Germany, 9University of Leipzig, Leipzig, Germany, 10Pharmazentrum Frankfurt/ZAFES, Infection Immunology, Frankfurt, Germany, 11Cincinnati Children’s Hospital Medical Center, Cincinnati, United States, 12Institute for Pathology, Martin-Luther University Halle, Halle, Germany, 13German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany.

Regulatory T cells (Treg cells) are important for the prevention of autoreactivity and lately, their role in maintaining tissue homeostasis has been demonstrated. They exert their function via different suppressive mechanisms including soluble factors. However, how Treg cells exert their function in tissue specific environments is often unknown. Here, we show that T cell specific expression of hydroxydegradase (HPGD), which catalyzes prostaglandin E, (PGE) into 15- keto-PGE, enforces a new suppressive mode of action through accumulation of the PPAR-γ ligand 15-keto PGE, PPAR-γ-dependent HPGD expression acts as the critical molecular link between prostaglandin metabolism, adipose tissue (AT)-associated Tregs cell function, and maintenance of AT homeostasis. In mice, loss of HPGD results in increased numbers of non-functional Treg cells, which accumulate in visceral adipose tissue resulting in increased local inflammation and systemic insulin resistance. In line with this, we could show that in type 2 diabetes (T2D) patients the peripheral body fat mass is reduced and exhibited decreased HPGD expression, indicating that HPGD might be involved in the development of T2D in humans. These data support that HPGD is critical for Treg cell functionality both in vitro and in vivo and that HPGD acts as a novel tissue- and context-dependent suppressor mechanism by Treg cells to maintain adipose tissue homeostasis.

P.C1.01.20 T-cell dynamics in Heligmosomoides polygyrus infection revealed by a new Timer approach C. B. Duke1, D. Bending1, M. E. Sekik1, M. O’ro1, 1Imperial College London, London, London, United Kingdom, 2University of Birmingham, Birmingham, United Kingdom.

Heligmosomoides polygyrus (H. polygyrus), an intestinal nematode parasite, induces regulatory T-cells (Tregs) to persist within the murine host through secreted effector proteins. Here, we determine the time-course and character of T-cell responses to H. polygyrus infection in a mice model that displays a delay of Fopx increases in CD4+ T-cells by day 7 post infection. These cells appear to have sustained Fopx3 transcription, becoming Treg. At day 28 of infection, Fopx3 transcription is modestly re-instated in a population. We further analysed transcriptomic responses of Tocky populations by RNA-seq. In conclusion, two individual Treg responses appear to be occurring during H. polygyrus infection, depending on the phase. An initial, most likely parasite-mediated response is followed by a major response of Treg that may be important for tissue damage, subsequent damage superficial, of TCR signalling suggests a potential break in T-cell non-responsiveness during parasite migration.

Funding: BBSRC, MRC.

P.C1.02 Maintenance and local regulation of tissue specific immunity - Part 2 P.C1.02.01 Impaired immunomodulatory function of T-reg derived exosomes in RRMS patients M. Azimi1, M. Izadi2, 1Tehran University of medical science, Tehran, Iran, Islamic Republic of.

Introduction: Multiple sclerosis is an autoimmune disease which is characterized by neuroaxonal degeneration in central nervous system. Impaired function of regulatory T cells (Tregs) is believed to be an underlying pathogenic mechanism in MS. Tregs is able to release exosomes, which contain a considerable amount of protein and RNA. Exosomes are capable of transporting their content to other cells where the released content exerts biological functions. Here, we investigated whether Tregs-exosomes of RRMS patients or healthy controls might regulate the proliferation or survival of T lymphocytes.

Methods: Regulatory T cells derived from MS patients or healthy controls were cultured for 3 days and exosomes were purified from supernatants. Treg-derived exosomes were co-cultured with conventional T-cells (Tconcs). The percentages of Tconcs proliferation and apoptosis were measured. Result: Our findings showed that the percentage of proliferation suppression induced by exosomes in patients compared to healthy controls was 8.04±1.17 and 12.5±1.22, respectively. p-value=0.035. Moreover, the rate of Tconcs apoptosis by exosomes of MS patient was less than healthy controls (0.68±0.12 vs. 1.29±0.13; p-value=0.015). Overall, Treg-derived exosomes from MS patients and healthy controls suppressed the proliferation and induced apoptosis in Tconcs. However, the effect of MS-derived exosomes was significantly less than healthy controls.

Conclusion: Our results point to an alternative Treg inhibitory mechanism which might be important in immunopathogenesis of MS. Although, the cause of the exosomal defect in MS pathogenesis is unclear, manipulation of patients' T-reg derived exosomes to restore their suppressive activity might be considered as a potential therapeutic approach.

P.C1.02.02 The blockage of NMDAR decrease the inflammatory response of T helper cells in an animal model of Multiple Sclerosis W. N. Brandão1, A. C. Durão1, T. T. Braga1, C. Longo1, C. Polinário1, N. Ghadban1, J. L. Oliveira1, T. Marcoukas1, J. S. Feran1, 1Institute of Biomedical Sciences, São Paulo, Brazil, 2Faculty of Pharmaceutical Sciences - University of São Paulo, São Paulo, Brazil, 3Federal University of Paraná, Curitiba, Brazil.

Multiple sclerosis (MS) consists of an autoimmune disease that has its pathology due to an infiltration of immune cells in the central nervous system (CNS) promoting inflammation and death of resident cells. The factors that influence this disease are not fully understood, especially the effects of neurotransmitters on infiltrated cells. Data from our group shows that glutamate is involved in this pathology and the blockade of its receptor NMDA in mice decreases both disease and pathogenic T cells frequency in the CNS. However, it is not clear if this phenomenon is control over this, we deposited the spinal cord of another model a model of mice that have the NMDAR blocked only in the T lymphocytes, through the crossing of C57BL/6 CD4+ Cre mice with C57BL/6 Grin1 Fox. This result data shows a delay in the clinical scores of animals knockout for NMDAR in 7 lymphocytes. This result data shows a delay with lower production of proinflammatory cytokines (IFN-γ and IL-17) by the leukocytes present in the draining lymph nodes during the antigen presentation period (7 days after immunization). In addition, we find a smaller amount of T helper lymphocytes present in these organs, which may be due to a lower proliferative capacity of these cells. Lastly, our data point to a direct effect the downstream of the NMDAR on the T helper lymphocyte response. Financial support CAPEX
Influence of Invariant chain and HLA-DM on the HLA-DR3 peptide constitution

R. Farrochi, Y. Arbabzadeh, C. Guiraud, L. Labrousse, V. Casar, M. Carrascillo, D. Jarajemadadi

1Department of Biomedicine and Biotechnology, Autonomous University of Barcelona, Cerdanyola del Vallés, Spain; 2CSIC IAP Proteomics Laboratory, CSIC-IBIB, Cerdanyola del Vallés, Spain.

Genetic wide-association studies (GWAS) have pinpointed a high risk to several autoimmune diseases including type 1 diabetes, associated to certain HLA-class-II haplotypes, of which DR alleles HLA-DRB1:04:01 (DR4) and HLA-DRB1:03:01 (DR3) stand out. In a previous study, our group showed the influence of the Invariant Chain (II) and HLA-DM on the DR3-peptide repertoire using a rat insulinoma cell line (RINm5F) transfected with DRB1, II, and HLA-DM human genes. A similar panel of four transfected cell lines (RIN-DRD2II, -DRD3II, -DR3II, and -DR3II and -DR3II was used to dissect the individual effects of these chaperones on the DR3-peptide. The results showed that both II and DM influenced most of the peptide characteristics, including peptide length distribution, DR3 motif compliance, predicted binding affinity and preference for endocytotic degradation. In contrast, the subcellular origin of the peptides represented in the DR3-associated peptide repertoire was independent of chaperone expression. Thus, one third of the peptides from the DR3 repertoire from all the transfected cells derived from cytosolic degradation, i.e. were either from cytosolic or nuclear proteins. The DR3 repertoire from a homoygous human B-cell line showed around 15% of such peptides. These results differ from those from the DR4 transfecants, where the frequency of cytosol-degraded peptides was only high in the HLA-DR4 single transfecants and the expression of II and DM normalized the subcellular origin distribution. The data thus suggested an allele dependent feature of the DR3-associated peptide repertoire.

Increased costimulatory molecule expression in thymic and peripheral B cells and a sensitivity to IL-21 in myasthenia gravis

M. HOCAHOGLU, B. OZCOK, S. YENTUR, O. DOGAN, Y. PARMAK, F. DEYMEER, G. SARUHAN-DIRESKENELI; 1Department of Pathology, Faculty of Medicine, University of Istanbul, Turkey.

15.00 Normal 0 21 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Normal Table"; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin-top:0cm; mso-para-margin-right:0cm; mso-para-margin-left:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Times New Roman"; mso-ascii-font-family:"Times"; mso-fareast-font-family:"Times"; mso-hansi-font-family:"Times"; mso-font-kerning:0pt;}

Molecular dissection of the genetic variants from 3,903 healthy and autoimmune individuals with different levels of autoantibodies (autoAbs) revealed two major genetic loci in HLA-DM and C11orf30 locus associated with breach of immune tolerance and autoantibody production in healthy and SLE.

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Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.C1.02.09 Circulating IL-10 producing B cells in CSF of neuroimmunological disorders patients
G. Moghreb1, M. beghyhi1, K. Bahrin1, S. ben Sassi2, S. Boto, M. R. Barboche1;
1Institut Pasteur de Tunis, Belvédère Tunis, Tunisia, 2Institut Pasteur de Tunis, tunis, Tunisia, 3Institut Pasteur de Tunis, Belvédère tunis, Tunisia, 4Institut Mongi Ben Hmidia de Neurologie De Tunis, Tunis, Tunisia.

Introduction/objective: Neuroimmunological disorders are a spectrum of diseases characterized by a chronic neuroinflammation causing neurologic lesions. The recurrent clinical course of the majority of those diseases suppose an interplay between self-reactive and immunoregulatory process. T and B cells are generally considered as effectors cells, but it is unclear that they are essential for inducing immune tolerance by regulating immune responses via IL-10 production. The aim of this work is to assess involvement of different cell subsets secreting IL-10 in the cerebrospinal fluid of patients with neuroimmunological disease. Material /Methods: Blood and cerebrospinal fluid (CSF) samples from patients with CNS inflammatory diseases were collected at time of clinical relapse. Ethical clearance and written consent were obtained for all of them. Isolated PBMCs and CSF cells were immunostained with a combination of specific antibodies and then analyzed using cytometry. Results/ conclusion: Our results show IL-10 higher expression in CSF neuro-inflammatory diseases like Neuro-Behcet compared to neuro-autoimmune disorders. The presence of dermal CD8+ T-cells was evaluated by immunohistochemistry. Results: In peripheral blood of patients with vitiligo compared to healthy individuals, CD8+ T-cells demonstrated an increased frequency of Granzyme B and CXCR3. Additionally, levels of circulating pro-inflammatory cytokines (IL-2, IL-6, TNF-α, IFN-γ, IL-18, IL-8) and chemotactants (CCL9, CCL10, CCL11) were measured using a multiplex assay. The presence of dermal CD8+ T-cells was evaluated by immunohistochemistry. Results: In peripheral blood of patients with CD8+ T-cells as compared to healthy individuals, CD8+ T-cells demonstrated an increased frequency of Granzyme B and CXCR3. Additionally, levels of circulating pro-inflammatory cytokines (IL-2, TNF-α, IL-18, IFN-γ and IL-8), and chemokines (CCL9, CCL10) was enhanced. An increased presence of epidermal CD8+ T-cells was demonstrated in the perilesional skin of patients with vitiligo. Conclusion: Patients with vitiligo had higher levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-18, IFN-γ) than controls. The increased levels of IFN-γ possibly caused upregulation of CCR3 in CD8+ T-cells, and the concomitant increase in the levels of chemokines, CCL9 and CCL10 facilitated the migration of CD8+ T-cells to the dermal milieu which may be responsible for the destruction of melanocytes, a hallmark of the disease. Funding: •Dept. of Science & Technology (DST). Govt. of India & Govt. of West Bengal •Fellowship INSPIRE Programme DST, Govt. of India.
POSTER PRESENTATIONS

P.C1.02.14
Differentiation analysis of patients with antibodies related to systemic sclerosis
M. San José-Cascao1, A. Pérez Linaza2, R. de la Varga Martínez2, E. Velazquez2, F. Medina Varo2, C. Rodriguez2
1UGG, Hematology, Immunology and Genetics, Hospital Universitario Puerta del Mar, Cadiz, Spain; 2Hospital Universitario Puerta del Mar, Cadiz, Spain; 3Hospital Universitario Virgen del Rocío, Sevilla, Spain; 4Hospital Dr. Arturo Ollavia, Buenos Aires, Argentina.

Abstract: Systemic sclerosis (SSc) is an autoimmune disease characterized by cutaneous and visceral fibrosis associated to small- vessel vasculopathy. There is evidence that both capillary anomalies and the course of disease are associated to the presence of specific autoantibodies (Ab) such as anti-centromere Ab (CENP-Ab) and anti-topoisomerase I Ab (Scl-70). Objective: Our aim was to evaluate the diagnostic and prognostic value of CENP and Scl-70 Ab in patients diagnosed with SSc from our hospital.

Patients and methods: Patients with specific Ab of Scl-70 were retrospectively selected from January-2012 to December-2017 (n=70). Clinical and laboratory data were collected and patients were classified according to ACR/EULAR 2013 criteria. The association between Ab and clinical parameters were assessed in the group of patients diagnosed with SSc (n=27).

Results: Among 70 patients, 58 had CENP-Ab and 12 had Scl-70 Ab. 2/3 of patients with SSc presented the limited form and the most frequent clinical manifestations were Raynaud phenomenon, digestive complaints and telangiectasias. Digital ulcers were observed in 9% of CENP-Ab vs 100% of Scl-70 positive patients (p<0.05, q2). Lung involvement was present in 54% of patients with CENP-Ab vs 100% of patients with Scl-70 (p<0.05, q2). Patients with anti-Scl-70 had higher levels of ESR than those with CENP-Ab (p<0.05, q2).

Conclusion: The presence of Scl-70 was associated with more frequent lung involvement and digital ulcers and higher levels of ESR, whereas CENP-Ab appeared in limited SSc. Therefore, anti-Scl-70 Ab are laboratory markers for identifying SSc patients with worse prognosis.

P.C1.02.15
Role of neutrophils in the induction of autoantibodies in a murine model of Epidermolysis bullosa acquisita
J. Tillmann1, M. R. Raman1, K. Petroni1,2, R. Parente1, M. Gabbii1, B. Battazzii1, A. Mantovanii1, A. Inferraezi1,2
1Department of Biomedical Sciences, Humanitas University, Pieve Emanuele (Milano), Italy; 2IRCNS-Humanitas Clinical Research Institute, Rozzano, Italy; 3IRCCS “Mario Negri” Institute for Pharmacological Research, Milano, Italy.

Epidermolysis bullosa acquisita (EBA) is a skin blistering disease caused by autoantibodies (auto-Abs) against collagen type VII (Col7), one of the major components of anchoring fibrils. These auto-Abs trigger complement activation via the alternative pathway (AP) and interact with the cellular receptors C5aR1 and C5aR2 (CD88) on neutrophils, which are found in high concentration in blister fluids and skin. C5aR1+ neutrophils are critical in mediating skin blistering in EBA patients. In contrast to previous reports that FcγRIII and FcγRIV are equally important for activating neutrophils. Our findings identify the C5a/C5aR1 axis as a critical driver of EBA. C5aR1

P.C1.02.16
Highly inflammatory multiple sclerosis patients show an increase in B cell cytokine production in cerebrospinal fluid
A. Vieda Velarde1, E. Rodríguez Martí1, L. Costa-Frasses1, Y. Aladó1, S. Sainz de la Maza1, M. Espié1, S. Medina1, N. Villarrubia1, E. Monreal1, J. Álvarez-Cermeño1, R. Rodó1, L. Urquiza1, A. Pérez Linaza1,2
1Ramón y Cajal University Hospital, Madrid, Spain; 2Getife University Hospital, Madrid, Spain.

Introduction: B cells play a central role in humoral immunity via antibody production but have also antibody-independent functions. There is an increasing appreciation of the importance of tissue-resident immune cells in generating local immune responses. To date, the question of whether B cells reside in non-lymphoid organs has received little attention.

Patients and methods: We analyzed the number, phenotype and clonality of B cells in human kidneys that were perfused to remove circulating cells, and in splenic tissue obtained from the same donor (N=10, median age 56 years (range: 23-80)). Percoll single cell suspensions from homogenized organs were analyzed using a 35-marker mass cytometry panel, and B cells were also sorted for RNA-sequencing.

Results: The frequency of B cells in both the renal cortex and medulla was lower than that observed in spleen (8.8% and 5.8% vs. 26%, P<0.005). The renal cortex harbored five times more B cells per gram of tissue than medulla (P 0.02). Both renal cortex and medulla were significantly enriched for non-naïve B cells compared with spleen. BCR analysis showed CDR3 repertoire differences between kidney and spleen suggesting a specific antigenic exposure. Conclusion: Our studies show that under homeostatic conditions, the extravascular compartment of human kidneys harbors antigen-experienced B cells, mirroring studies of murine tissue-resident T cells. These kidney B cells may play a role in local immune defense or contribute to immunopathology and further studies on diseased tissues and murine models are underway.

P.C1.02.17
Age related macular degeneration and innate immunity: molecular crosstalk between the complement system and the long pentraxin 3
A. Del Rocío1,2, A. Pérez Linaza1, A. Boscá1, M. R. Raman1, K. Petroni1,2, R. Parente1, M. Gabbii1, B. Battazzii1, A. Mantovanii1, A. Inferraezi1,2
1Laboratory of Molecular Biology, Molecular Immunity Unit, University of Cambridge, United Kingdom; 2Irccs-Humanitas Clinical Research Institute, Rozzano, Italy.

Age related macular degeneration (AMD) is a leading cause of vision loss in adults older than 60 years. FH is the long pentraxin 3 (PTX3), a soluble pattern recognition molecule involved in innate immunity, tissue homeostasis and inflammation. PTX3 is locally produced in the retina following ischemia and in response to oxidative stress. FH is the long pentraxin 3 (PTX3), a soluble pattern recognition molecule involved in innate immunity, tissue homeostasis and inflammation. PTX3 is locally produced in the retina following ischemia and in response to oxidative stress. Importantly, FH-C3b interactions and their effect on AP activation. In solid phase and surface plasma resonance binding experiments we found that PTX3 specifically recognizes C3b, and this interaction is strengthened by FH. Importantly, C3b could not trigger AP activation when bound to PTX3. We extended these investigations to ARPE-19 cells under either oxidative or inflammatory conditions, to model the human disease in vitro. These findings point to a novel mechanism of complement regulation by PTX3 with potential implications in the etiopathogenesis of AMD.

P.C1.02.18
The relevance of the C5a/C5aR1 axis in production of auto-antibodies in the murine immunization model of Epidermolysis bullosa acquisita
A. del Rocío1,2, A. Pérez Linaza1, A. Boscá1, M. R. Raman1, K. Petroni1,2, R. Parente1, M. Gabbii1, B. Battazzii1, A. Mantovanii1, A. Inferraezi1,2
1Laboratory of Molecular Biology, Molecular Immunity Unit, University of Cambridge, United Kingdom; 2Irccs-Humanitas Clinical Research Institute, Rozzano, Italy.

Epidermolysis bullosa acquisita (EBA) is a skin blistering disease caused by autoantibodies (auto-Abs) against collagen type VII (Col7), one of the major components of anchoring fibrils in the dermal-epidermal junction. Previously, we found that deficiency of CslA1 protects from disease development in an antibody-transfer model of EBA, demonstrating an important role for CslA1 in the effector phase of the disease. Here, we show the importance of CslA1 in an immunization model of EBA and redefine the view on the contribution of Fcγ-receptors (FcγR) to disease development. The active EBA mouse model is induced by subcutaneous immunization with Col7. Mice were scored and induction of Col7-specific auto-Abs was analyzed in wild type (WT) and CslA1-deficient (CslA1-/-) mice, biweekly. Additionally we determined IgG pathogenicity using ROS-release-assay and assessed IgG Fc glycosylation, which defines pro- or anti-inflammatory properties of IgG. We observed first symptoms after 4 to 6 weeks in WT mice, whereas CslA1-/- mice were protected from EBA. CslA1-/- showed significantly decreased levels of pro-inflammatory agalactosylated auto-Abs compared to WT. Further, analyzing IgG pathogenicity using ROS-release-assay we found that in contrast to previous reports that FcyRIIIa and FcγRIIV are equally important for activating neutrophils. Our findings identify the C5a/C5aR1 axis as a critical driver of EBA not only in the effector but also in the initiation phase shaping a pathogenic Ab response. Furthermore, our findings show that the previously assumed exclusive dependency of FcγRIIV is not valid anymore and needs to be re-evaluated.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 305
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.C1.02.19
Impact of CD38 on the activation and function of regulatory T-cells in autoimmune diseases
M. Yang, S. Horstmann, A. A. Ettinger, C. Speake, A. E. James, K. Heraldo, M. J. Mamula;
Yale School of Medicine, New Haven, United States, Banenura Research Institute, Seattle, United States.

Introduction: Inflammation and oxidative stress in pancreas amplifies various post-translational modifications (PTMs) on self-proteins. The loss of immune tolerance to PTMs within the stressed islets subsequently impacts the autoreactive T cell epitope repertoire and contributes to the destruction of insulin-producing beta cells in T1D. However, the mechanism of carboxylation and citrullination, common PTMs found in stressed islets, modulates immunogenicity and islets functions is still unclear.

Materials and Methods: Mass spectrometry was performed to map the PTM sites. Humoral responses and immunogenicity to modified islet proteins were evaluated by ELISA and HLA tetramers, respectively. Finally, proinsulin and insulin secretion upon glucose stimulation was examined in human islets in the context of beta cell PTMs.

Results: We identified six carbonyl residues within CD38 and 42 citrullination modifications within glucokinase. Carboxylated-CD38 is amplified in stressed islets coincident with decreased glycosyl-stimulated insulin secretion and altered proinsulin to insulin ratios. Autoantibodies against both proinsulin and glucokinase arise in human T1D patients. Likewise, CD+ T cells specific for citrullinated glucokinase are present in the circulation of T1D patients. Mature insulin is stored in the form of Zn-containing hexamers until secretion. Interestingly, anti-glucokinase antibodies correlated with anti-ZnT8, but not with other T1D autoantibodies (Insulin, GAD, IA-2).

Conclusions: In beta cells, glucokinase acts as a glucose sensor to initiate glycolysis and insulin signaling. P48 is critical for proinsulin/insulin folding. Our studies implicate these crucial enzymes as biomarkers, providing new insights into creating autoantigens and define the impact of P48 on the biological function of beta cells in T1D.

P.C1.02.20
Human intestinal mucosa contains a large population of resident memory T cells
1Department of Pathology and Centre for Immunology Regulation (CIIR), Oslo University Hospital – Rikshospitalet, Oslo, Norway, Core Facility Flow Cytometry, Biomedical Center, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany, 2Department of Immunology and Centre for Immune Regulation (CIIR), Oslo University Hospital – Rikshospitalet, Oslo, Norway, 3K.G. Jebsen Coeliac Disease Research Centre, Oslo University Hospital – Rikshospitalet, Oslo, Norway, 4Department of Gastrointestinal Surgery, Oslo University Hospital – Rikshospitalet, Oslo, Norway, 5Department of Transplantation Medicine, Section for Transplant Surgery, Oslo University Hospital, Rikshospitalet, Oslo, Norway, 6Department of Gastroenterology, Oslo University Hospital - Rikshospitalet, Oslo, Norway.

Recent studies in mice have described resident memory CD8 T-cells (Trm) as key immune mediators of long-lasting protective responses in non-lymphoid tissues. The gut is a large immune organ and one of the main sites of patterned entry. However, our knowledge about CD8 Trm in the human gut is very limited. Here we examined the replacement of CD8 T-cell subsets in transplanted small intestine (SI) by flow-cytometric analysis of HLA-I mismatched donors. We found that 60% (n=15) of CD103+ CD8 T-cells both in the epithelium and lamina propria (LP) were of donor origin one year after transplantation, whereas LP CD103- CD8 T-cells were rapidly exchanged (15%, n=15). Single-cell TCR sequencing of donor CD103+ CD8 T-cells from transplanted SI revealed that a significant fraction of expanded TCR clonotypes at baseline were maintained one year after transplantation. The persistent CD103+ CD8 T-cells were CD69+ KLRG1- CD127- CD28+ PD1+, and this distinctive phenotype was progressively acquired by the incoming recipient CD8 T-cells. LP CD103+ CD8 T-cells showed an additional substantial clonal expansion. Moreover, we found mutually overlapping overlap between CD103+ and CD103- KLRG1- CD8 subsets in LP, suggesting that CD103+ CD8+ Trm might derive from CD103- KLRG1- precursors. CD103+ CD8 T-cells expressed lower levels of preformed cytotoxic granules but produced more cytokines than the CD103- counterpart. In summary, we provide definitive evidence that most human intestinal CD103+ CD8 T cells persist over long periods of time and show immunogenic, phenotypic and functional characteristics of murine CD8 Trm.

P.C1.03 Maintenance and local regulation of tissue specific immunity - Part 3

P.C1.03.01
CD39/CD73 implication in neuroimmunological inflammatory disorders
k. BAHRIN1, M. Belghribi1, O. Maghreb1, S. Ben saadi1, S. Belafi, M. Barbouche2;
1Insitut pasteur de Tunis, Tunis, Tunisia, 2Institut Mongi ben Hamida de Neurologie De Tunis, Tunis, Tunisia.

Introduction/Objective: Treg cells can be divided in two subsets based on the expression of CD39 an ectonucleotidase that catalyzes the conversion of pro-inflammatory extracellular ATP to adenosine which present a regulatory effect. CD39+ Treg cells do not express CD73 and possibly contribute to the maintenance of CD39 expression in vivo. The purpose of our study is to determine the expression of CD39 in the blood and cerebrospinal fluid (CSF) of Multiple sclerosis and Neuro-behçet disease to determine the role of these enzymes in these two disorders.

Material and methods: We quantified, using quantitative RT-PCR, the mRNA expression of IL-10, IL-14, GATA3, Foxp3, CD39 and CD73 in the PBMC and CSF of 21 patients with relapsing remitting multiple sclerosis (RRMS, 19 patients with Neuro-Behçet disease (NBD) and 22 healthy controls. CD39 and CD73 in blood and CSF were studied simultaneously with flow cytometry.

Results: Our results show no significant difference in the expression of IL-4, IL-14, GATA3 and Foxp3 mRNA in the blood and CSF of the three studied groups. Concerning CD39 expression, we revealed a significant decrease of CD39 regulatory cells expressing Foxp3 and CD10 in PBMC of RRMS compared to NBD (p<0.05). Surprisingly, in the CSF we detected a high level of CD39 in RRMS and NBD patients (p<0.001) compared to controls. CSF NBD CD39 cells aren’t associated with regulatory markers. However in half of RRMS patients, CD39 is associated with IL-10 and CD73. These results demonstrate a differential expression of CD39 depending on the inflammatory CNS Stake.

P.C1.03.02
IL-17 producing INKT cells correlate positively with disease activity DAS28-ESR in rheumatoid arthritis
K. CHOUDHURY, D. K. Mitra1, U. Kumar1, S. Dey2;
1All India Institute of Medical Sciences, Delhi, India, 2Albert Einstein College of Medicine, USA., Newyork, United States.

Rheumatoid arthritis (RA) is well established autoimmune disease mediated through various pathogenic T cells. INKT cells constitute 0.5% of total NKT cells. The role of INKT cells in RA animal model (collagen induced arthritis; CIA) is shown to be protective by IL-10 production. On contrary they are also shown to be secretor of IL-17 which is pathogenic to RA.

There is no direct evidence of human study which shows the precise role of IL17 in disease pathogenesis or amelioration. So we intend to study CD161 restricted INKT cells in PBL and SF of rheumatoid arthritis patients. We have recruited treatment naive active RA patients with DAS28-score<3 (n=25). Cells derived from peripheral blood and synovial fluid was stimulated with alpha-galactosylceramide (alpha-gal) for 48 hours to measure cytokine production and proliferation. We have observed higher frequency of IL-17+ INKT cells as well as CD1d restricted INKT cells in RA SF compared to autologous PBL (p<0.002) and positively correlates with DAS28-ESR (R=0.79). CD3+CD161+ NKT cells were also higher in RA SF. Moreover alpha-galcer stimulation up regulated IL-17+ INKT cells instead of IL-10 in RA SF derived cells. RA synovial CD161 restricted INKT cells are polarized towards IL-17 production instead of IL-10 upon alpha-galcer stimulation. IL-17+INKT cells proliferates more in RA SF upon alpha-galcer stimulation and also correlates with DAS28 ESR. Taken together these findings hint towards pathogenic role of INKT cells in RA.

P.C1.03.03
TIGIT expression identifies gut-homing effector T cells with immunomodulatory properties that are deregulated in inflammatory bowel disease patients

Inflammatory bowel disease (IBD) is characterized by intestinal infiltration of pathologic effector T-cells. The defects driving loss of normal T-cell regulation in IBD remain undefined. We co-cultured murine gut-homing monocytes and human intestinal tissue 40-50% of gut resident CD38+CD8+ effector T-cells express CD161+immunglobulin and ITIM domain (TIGIT), an inhibitory receptor capable of modulating dendritic cell (DC) and T-cell function. In peripheral blood of healthy individuals, TIGIT expression was enriched on gut-homing antibodies (IgA, IgG, IgM). Interestingly, anti-glucokinase antibodies correlated with anti-ZnT8, but not with other IBD autoantibodies (Insulin, GAD, IA-2).

Conclusions: In beta cells, glucokinase acts as a glucose sensor to initiate glycolysis and insulin signaling. P48 is critical for proinsulin/insulin folding. Our studies implicate these crucial enzymes as biomarkers, providing new insights into creating autoantigens and define the impact of P48 on the biological function of beta cells in T1D.
Granular cornal dystrophy type 2 (GCD2) is caused by a point mutation (R124H) in the transforming growth factor-β (TGF-β) induced gene (TGFBI). The accumulation of the protein TGFBIp in the corneal stroma results in the disruption of corneal transparency and finally in a loss of vision. However, the mechanisms underlying the accumulation of TGFBIp and the consequences are poorly understood. In this study, we showed that in GCD2 patients TGFBI expression was reduced upon IL-7 treatment in corneal fibroblasts, suggesting that impaired IL-7 expression in patients with GCD2 affects disease pathogenesis via a failure to control TGFBI expression. Interestingly, the interleukin between TGF-β and IL-7 was regulated by the RANKL/RANK signaling cascade. We also found that IL-7 regulates the expression of a membrane-type matrix metalloproteinase (MT-MMP), which plays a crucial role in migration and neovascularization in the cornea via the RANKL/RANK axon. Taken together, we demonstrated that the RANKL/RANK axis regulates TGF-β and TGFBI expression via IL-7-mediated MT-MMP regulation in corneal fibroblasts; these findings improve our understanding of the pathogenesis of GCD2.

**P.C1.03.05**

**Mucosal lesions derived from oral cavity identified as lichen planus express high levels of IFN-γ and IL8**


1Hospital Israelita Albert Einstein – Instituto de Ensino e Pesquisa Albert Einstein, São Paulo, Brazil, 2(2) Centro de Especialidades Odontológicas/Estomatológicas – Unidade Alto da Boa Vista - Secretaria Municipal de Saúde de São Paulo, São Paulo, Brazil, 3(3) Instituto de Responsabilidade Social- Programas Governamentais - Hospital Israelita Albert Einstein, São Paulo, Brazil.

**Background:** Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disease that is Th1-mediated and affects skin and the oral mucosa with a variety of clinical presentations. Lesions are usually bilateral and are often sensitive or painful. Cutaneous lesions of Lichen Planus (LP) are self-limiting; however, oral lesions are chronic and rarely remissive. Interferon-gamma (IFN-γ) is an important cytokine involved in the regulation of local immune response in OLP, and it has been frequently studied in these lesions. Aims: Identify cytokines present in lesions suggestive of OLP and compare to the controls. The controls were a group of patients with non-specific inflammatory lesions (NSIL). Methods: Histopathological image analysis, immunohistochemistry and gene expression were performed in oral mucosal tissue derived from samples suggestive of OLP and controls. All samples were obtained after patient signed an informed consent approved under CAEE number 55057165530010068. Results: Our preliminary results demonstrated higher numbers of lymphocytes infiltrating OLP lesions compared to controls (p=0.025) and more T CD4 lymphocytes into epithelial tissue (p=0.006). In addition, the OLP samples showed a higher percentage of activated cells compared to controls (p=0.047). Regarding cytokine analysis, there were no significant differences in both NSIL and OLP groups, significant expression of IFN-γ and IL-33 in OLP groups compared to NSIL (p=0.001; p=0.026). Conclusion: The lesions characteristic as OLP demonstrated higher numbers of activated cytokines, and increased levels of IFN-γ and IL-33 which described them as more aggressive lesions than NSIL.

**P.C1.03.06**

**Impact of skin T cell secretome on the epidermal barrier during vitiligo**

C. Martins; 1, F. Lucchese; 1, A. Tabej; 1, J. Seneschal; 1, K. Boniface; 1

1Inserm U1035, BMGIC, Bordeaux University, Bordeaux, France; 2Department of dermatology and Pediatric Dermatology; National Reference Center of Rare Skin disorders, hôpital Saint-André, Bordeaux, France.

**Introduction:** Resident memory T cells have a key role in the development of chronic inflammatory dermatosis, such as vitiligo, the most common depigmenting disorder resulting from the loss of epidermal melanocytes. We previously showed that vitiligo skin is imprinted with resident memory CDB T cells producing elevated levels of the inflammatory cytokines IFNγ and TNFs, while displaying moderate cytotoxic activity. We further analyzed the cytokine secretion profile of skin T cell secretome and characterized its effect on the inflammatory response and melanocyte alteration. **Materials and Methods:** The cytokine secretion profile of skin T cells was analyzed by Flow cytometry and multiplex ELISA following T cell activation. In addition, primary cultures of normal human epidermal keratinocytes and melanocytes were stimulated in the presence or absence of skin T cell supernatants and expression of genes involved in the inflammatory response, and melanocyte function was assessed by real-time PCR. **Results:** We show that skin T cell secretome contributes to the development and amplification of the inflammatory response by increasing the expression of chemokines and factors involved in inflammation and T cell homing, such as CXCL9 and CXCL10, by epidermal cells; while inhibiting melanocyte function, leading to the loss of melanocytes. **Conclusion:** Our results highlight the importance of skin T cell secretome in vitiligo pathogenesis, and show the role of T cell soluble factors on both the loss of melanocytes, and on the increase of the inflammatory response through an effect on both keratinocytes and melanocytes.

**P.C1.03.07**

**The effect of the first and third trimester placentas secretory factors obtained from normal pregnancy on the tube-like structure formation by endothelial cells in presence of trophoblast cells**


1Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-Universität Mainz, Mainz, Germany, 2Inserm U1035, BMGIC, Bordeaux University, Bordeaux, France.

**Introduction:** Trophoblast cells (TC) interact with endothelial cells (EC) in the uteroplacental contact area. Effects of placenta-derived factors upon local interactions between these cells remain unclear. The aim of the research was to evaluate the influence of placental factors upon formation of tube-like structures by EC in presence of TC. **Materials and Methods:** EC line EA.Hy926 and TC line JEG-3 were used in the experiment. Placentas were obtained after an elective pregnancy termination of normal 1st-trimester (9-11 weeks, group 1) pregnancy and after caesarean delivery of normal 3rd-trimester (38-39 weeks, group 2) pregnancy. EC and TC were cultured in 24-well-plates pretreated with Matrigel (BD, USA) in the presence of placental conditioned media (CM) with 2.5%FBS for 24 hours (37°C, 4.5%CO2). Control wells contained DMEM, 2.5%FBS without TC. Then we assessed the number and the length of formed tube-like structures using AxioObserver.Z1 microscope and program AxioVision. Statistical analysis was performed using Mann-Whitney test. Results: In the presence of CM of placentas of both groups we observed an increase in the number and a reduction in the number of tube-like structures formed by EC in the presence of TC, comparing with a spontaneous level of their formation. Also the same parameters in the presence of CM of placentas of group 2 were different from the parameters of group 1. Conclusion: Secretory factors contained in the CM of placentas change the characteristics of the vascular network formed by EC in the presence of TC.

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**P.C1.03.08**

**IL-17A signaling in IMQ-induced psoriasis-like dermatitis and host defense against Staphylococcus aureus**

S. Moos, T. Regen, I. Prinz, C. Reinhardt, K. Bitschar, L. Bleuf, B. Schickert, C. Woltz, A. Diefenbach, A. Waisman, F. C. Kurusch; 1

1Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-Universität Mainz, Mainz, Germany, 2Hannover Medical School, Institute of Immunology, Hannover, Germany, 3CHU, University Medical Center of the Johannes Gutenberg-Universität Mainz, Mainz, Germany, 4Dermatology, University Hospital Tübingen, Tübingen, Germany, 5Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany, 6Charité, Berlin, Germany, 7Department of Dermatology, Heidelberg University Hospital, Heidelberg, Germany.

IL-17A is the signature cytokine of Th17 cells and plays an important role in the development of many autoimmune and inflammatory diseases such as psoriasis and in host defense against microbial organisms. The IL-17 response of Th17 cells and especially of IL-17A-producing γδ T cells is crucial for mice to fight bacterial infections. Among the 6 members of the IL-17 family, IL-17A has the greatest homology to IL-17A. Moreover, IL-17A and IL-17F signal either as homo- or as heterodimers through a dimeric receptor composed of IL-17A and IL-17F. We used IL-17A full knockout mice or mice with cell type specific deficiencies for IL-17A to delineate which cell type needs to respond to IL-17 in order to develop immunomodulated dermatitis mimicking human psoriasis. Using this approach, we could clearly define keratinocytes as cell population necessary to respond to IL-17. Additionally to the mentioned IL-17A deficient mice, we also analyzed IL-17A cytokine double knock out mice. We found expanded populations of Rorγt expressing γδ T cells in the IL-17 signaling deficient mice. Furthermore, we could show that IL-17 signaling is important for defense against Staphylococcus aureus as the signaling deficient mice developed severe lesions composed of the coagulase positive bacterium. This work was funded by the DFG (CRC-TR156).
dcs are crucial for t-cell activation and tlo maintenance. thus, we hypothesized that dc subsets and t-cells are altered in ipah patients. tertiary lymphoid structures (tlos) in ipah lungs indicate a role for immune cell activation. in tlos, amongst other cell-types, t-cells and dendritic cells (dcs) are present, where pulmonary arterial hypertension (pah) is a cardiopulmonary disease characterized by high pulmonary arterial pressures, in which idiopathic pah (ipah) is the most common. d. van uden, m. van nimwegen, t. koudstaal, p. heukels, j. a. van hults, k. a. boormars, r. w. hendriks, m. koel; department of pulmonary medicine, erasmus mc, rotterdam, netherlands. pulmonary arterial hypertension (pah) is a cardiopulmonary disease characterized by high pulmonary arterial pressures, in which idiopathic pah (ipah) is the most common. t. koudstaal, p. heukels, j. van hults, a. boormars, w. hendriks, m. koel; department of pulmonary medicine, erasmus mc, rotterdam, netherlands.

p.c1.08.14

absence of stat1β increases th1 differentiation and exacerbates concanavalin a-induced hepatitis

a. puga, k. meisel, c. lasnig, s. macho-maschler, m. parnin, d. hainberger, w. ellmayer, m. müller, b. straib; university of vienna, vienna, austria, institute for immunology, medical university of vienna, vienna, austria.

signal transducer and activator of transcription 1 (stat1) is a transcription factor that is crucial for gene regulation in response to all types of interferons (ifn). stat1 has a crucial role in immunity against both men and mice: loss-of-function mutations cause severe autoimmune diseases. alternative splicing generates two stat1 isoforms: the full length stat1a and the truncated stat1b isoform, which lacks the c-terminal transcriptional activation domain and has compromised transcriptional activities. using stat1-/- knockin mice (i.e. mice that only express the stat1a isoform) we show that stat1b has important regulatory functions in t cells. cd4+ and cd8+ t cells, but not other cell types, from stat1b-/- mice have increased levels of stat1a compared to the levels of both stat1a isoforms in t cells from wild-type mice. in line with the crucial role of stat1 in th1 differentiation, stat1b-/- cd4+ t cells had increased levels of ifnγ and produced considerably more ifnγ upon activation compared to wild-type cells. to investigate whether stat1b is required to limit excessive ifnγ production in vivo, we challenged mice with concanavalin a, an experimental model for autoimmune hepatitis. stat1b-/- mice had exacerbated liver damage and higher systemic levels ifnγ compared to wild-type mice. currently, we investigate whether cd4+ t cells or nk t cells, the main drivers of cona-induced hepatitis, cause the increased ifnγ levels in stat1b-/- mice. in addition, we investigate th1 cell differentiation during viral infections. supported by the austrian science fund (fWF, K1212, SFB-F6101 and SFB-F6106).

p.c1.08.13

immunomodulatory effect of local complement regulators on retinal pigmented epithelium cells

n. schäfer, v. enzmann, d. pauly; department of ophthalmology, university hospital, regensburg, germany.

retinal complement activity has so far been related to macrophages or to the influence of liver-derived complement components. here we describe for the first time a liver-independent retinal complement system.

different cell types were isolated by immunomagnetic separation from murine and human retinas. expression analysis of specific cell markers, housekeeping and complement component genes was performed by qrt-pcr and western blot.

all isolated cell populations expressed c1s, c3, cfb, cfz, cfr and cfi. strikingly, we distinguished retinal cell types which mainly showed inhibiting complement transcripts from cell populations which synthesized mostly complement activating mRNAs. in vivo, mice showed an age-related change in cell type-specific complement expression levels towards a pro-inflammatory profile. in contrast to previously described protective effects of olaparib in oxidatively stressed retinae, we observed a more pronounced change towards pro-inflammation in olaparib-treated than in untreated arpe-19 cells.

our results show for the first time a functional link between oxidative stress, complement receptors, pro-angiogenic and -inflammatory responses of arpe-19 cells. these effects suggested an oxidative stress-associated mechanism of Csr2a-regulation in arpe-19 cells in connection with upregulated intracellular proteases.
POSTER PRESENTATIONS

Therapeutically, in-depth characterization of DCs and T-cells was performed in peripheral blood mononuclear cells (PBMCs) of IPAH patients and healthy controls (HCs) using 14-color flow cytometry. The proportion of total conventional DCs (cDCs) was decreased in IPAH patients compared to HCs, in which the percentage of type 1 cDCs (cDC1s) increased and cDC2s was unaltered. Furthermore, decreased percentages of the recently identified T-cell-stimulatory AS-DCs were found in IPAH patients compared to HCs. Strikingly, no differences were observed in memory T-cell proportions, however IPAH T-cells showed reduced capacity to produce interferon (IFN)-γ, interleukine (IL)-17 and IL-6. In conclusion, peripheral DCs and T-cells are altered in IPAH patients, wherein cDCs and AS-DCs are decreased. As cDCs and AS-DCs are potent T-cell stimulators, this could suggest homing towards lung TLOs. Furthermore, the reduced capacity of IPAH T-cells could be a consequence of ii) homing towards TLOs or ii) a negative feedback-loop due to a high pro-inflammatory cytokine milieu present in IPAH patients. These data indicate that immune cell activation could play a role in IPAH pathology.

Grant: Dutch heart foundation (2016/0702)

P.C1.03.15

RORγT inhibition selectively targets pathogenic human iNK and γ-T cells enriched in Spondyloarthritids while preserving IL-22 responses

K. Venken1,2, M. Leoblad1, P. Jacques1, T. Decroly1, J. Coudeyns1, K. Hoyt3, V. Van Gassen4, J. Wahle3, Y. Saeys2, G. Nabutsky1, D. Elewa1,2

1Institute of Laboratory Medicine, Ghent, Belgium; 2IVB Inflammation Research Center, Ghent, Belgium; 3Research and Development Boehringer-Ingelheim, Ridgefield, CT, United States.

In conclusion, peripheral DCs and T-cells are altered in IPAH patients, wherein cDCs and AS-DCs are decreased. As cDCs and AS-DCs are potent T-cell stimulators, this could suggest homing towards lung TLOs. Furthermore, the reduced capacity of IPAH T-cells could be a consequence of ii) homing towards TLOs or ii) a negative feedback-loop due to a high pro-inflammatory cytokine milieu present in IPAH patients. These data indicate that immune cell activation could play a role in IPAH pathology.

Grant: Dutch heart foundation (2016/0702)

P.C1.03.16

Ipilimumab targets CD27/CD70 costimulation in CD4+ T cells

C. Weise1, J. Pezoldt2, K. Loser1

1Institute of Laboratory Medicine, Ghent, Belgium; 2IVB Inflammation Research Center, Ghent, Belgium.

In conclusion, peripheral DCs and T-cells are altered in IPAH patients, wherein cDCs and AS-DCs are decreased. As cDCs and AS-DCs are potent T-cell stimulators, this could suggest homing towards lung TLOs. Furthermore, the reduced capacity of IPAH T-cells could be a consequence of ii) homing towards TLOs or ii) a negative feedback-loop due to a high pro-inflammatory cytokine milieu present in IPAH patients. These data indicate that immune cell activation could play a role in IPAH pathology.

Grant: Dutch heart foundation (2016/0702)

P.C1.03.17

IL-27 controls Treg persistence and differentiation in chronic inflammatory diseases

M. Zoub2, J. Pezoldt2, K. Loser1

1Institute of Laboratory Medicine, Ghent, Belgium; 2IVB Inflammation Research Center, Ghent, Belgium.

In conclusion, peripheral DCs and T-cells are altered in IPAH patients, wherein cDCs and AS-DCs are decreased. As cDCs and AS-DCs are potent T-cell stimulators, this could suggest homing towards lung TLOs. Furthermore, the reduced capacity of IPAH T-cells could be a consequence of ii) homing towards TLOs or ii) a negative feedback-loop due to a high pro-inflammatory cytokine milieu present in IPAH patients. These data indicate that immune cell activation could play a role in IPAH pathology.

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P.C1.03.18

The role of IL-27 on the balance of CD4+ T cells in health and chronic inflammatory disease

M. Zoub2, J. Pezoldt2, K. Loser1

1Institute of Laboratory Medicine, Ghent, Belgium; 2IVB Inflammation Research Center, Ghent, Belgium.

In conclusion, peripheral DCs and T-cells are altered in IPAH patients, wherein cDCs and AS-DCs are decreased. As cDCs and AS-DCs are potent T-cell stimulators, this could suggest homing towards lung TLOs. Furthermore, the reduced capacity of IPAH T-cells could be a consequence of ii) homing towards TLOs or ii) a negative feedback-loop due to a high pro-inflammatory cytokine milieu present in IPAH patients. These data indicate that immune cell activation could play a role in IPAH pathology.

Grant: Dutch heart foundation (2016/0702)
P.C1.03.20
Evolution of CD4+ T cell immunity to viral infection: analysis of systemic and local responses
B. J. Meckiff, H. M. Long; Institute of Immunology and Immunotherapy, Birmingham, United Kingdom.

BACKGROUND: Most studies investigating the immune response to viral infection have focussed on systemic immunity. However, mounting evidence suggests that immunity also involves localised non-circulating resident memory populations retained in specific anatomic compartments. To date, there is limited knowledge of resident memory CD4+ T cells induced by natural virus infection in humans.

Here we compare systemic CD4+ T cell immunity against the common herpesvirus Epstein-Barr virus (EBV) with the immune response at the site of infection, the tonsils. Clinical identification of Infectious Mononucleosis (IM) patients undergoing primary infection has enabled investigations of acute primary infection through to persistent carriage. A greater understanding of resident T cell memory in humans is paramount for the development of vaccine strategies.

METHODS: Matched PBMCs and tonsillar UMs were collected from patients suffering from acute IM or from healthy EBV carriers undergoing tonsillectomy. We used E/M-HCHI tetramers, representing a range of viral CD4+ T cell epitopes, to investigate the frequency and phenotype of virus-specific CD4+ T cells in both compartments.

RESULTS: Primary infection induces expanded populations of activated virus-specific CD4+ T cells in the blood of IM patients, but only low frequencies are detected in the tonsils. Following resolution of IM symptoms, EBV-specific CD4+ T cells are maintained in memory at a low frequency in both the circulation and at the site of infection. Furthermore, in long-term virus carriage, some virus-specific CD4+ T cells in the tonsil express resident memory-associated markers. Therefore EBV infection induces a population of virus-specific CD4+ T cells that are retained within the tonsil, with a different phenotype to circulating cells.

P.C1.04 Maintenance and local regulation of tissue specific immunity - Part 4

P.C1.04.01
Kidney disease alters the intestinal microbiota and regulates intestinal inflammation
K. Aono, K. Nishiyama, H. Nakajima, T. Takeuchi, Y. Azuma; Laboratory of Veterinary Pharmacology, Osaka Prefecture University Graduate School of Life and Environmental Science, Izumisano, Japan.

A number of organ-organ crosstalk is involved in homeostasis. Considering gastrointestinal symptoms are common in patients with renal failure, the aim of this study was to elucidate the relationship between gastrointestinal motility and gastrointestinal symptoms in chronic kidney disease. We performed studies with C57BL/6 mice with chronic kidney disease following 5/6 nephrectomy. Gastrointestinal motility was evaluated with ev responses of ileum and distal colon strips to electrical field stimulation. Feces were collected from mice and the composition of the gut microbiota was analyzed by 16S rRNA sequencing. Mice with chronic kidney disease after 5/6 nephrectomy showed the decreased number of fecal and this constipation is correlated with the suppressed contraction response in the ileum motility and decreased relaxation responses in the distal colon motility. Spermine, one of the uremic toxins, inhibited the contraction response in the ileum motility, but a kind of uremic toxins showed no effect on the relaxation responses in the distal colon motility. 5/6 Nephrectomy mice had the disturbed balance of gut microbiota. The motility dysfunction and constipation were cancelled by antibiotic treatments. Expression levels of IL-6, TNF-alpha, and INOS in 5/6 nephrectomy mice were increased in the distal colon but not in the ileum. In addition, macrophage infiltration in 5/6 nephrectomy mice were increased in the distal colon but not in the ileum. We found 5/6 nephrectomy to alter the gastrointestinal motility and cause constipation by changing the gut microbiota and colonic inflammation. These findings indicate that renal failure was remarkably associated with gastrointestinal dysregulation.

P.C1.04.02
Interleukin-19 protects Th2-mediated IBD model mice
Y. Azuma, T. Takeuchi; Laboratory of Veterinary Pharmacology, Osaka Prefecture University Graduate School of Life and Environmental Science, Izumisano, Japan.

IL-19 was originally found by sequence homology to IL-10, and is a member of the IL-10 family. However, little is known about the exact immunological role of IL-19 in the regulation of inflammatory bowel disease. Ulcerative colitis (UC) involves the superficial mucosal and submucosal layers of the colon and is driven by Th2 cytokines, such as IL-4, IL-5, and IL-13. In this study, we investigated the role of IL-19 in oxazolone-induced colitis which is useful for the study of the Th2 inflammatory response and resembles the symptom seen in UC. Bab/c genetic background IL-19 knockout mice (KO) were used. To presentize mice, 150 μl of a 4% oxazolone in 100% ethanol was applied in the shaved abdominal skin. Seven days after presentation, colitis was induced by intracolital administration with 100 μl of 3% of oxazolone in 50% ethanol using a plastic catheter. The colitis was evaluated by analyzing body weight loss and histology of the distal colon. IL-19KO showed severe weight loss. Histological analysis revealed that the distal colon in IL-19KO exhibited increased numbers of infiltrating cells and a general loss of tissue architecture. In IL-19KO, oxazolone treatment increased colonic MPO activity. Serum IgG levels and the number of circulating eosinophil were significantly elevated in IL-19KO. CD4+T-positive cells isolated from lymph node of IL-19KO produced elevated amounts of IL-4, IL-9, but not IFN-γ and IL-13. IL-19 plays an anti-inflammatory role in the Th2-mediated colitis model, suggesting that IL-19 may represent potential therapeutic target for reducing colonic inflammation.

P.C1.04.03
Anti-IL-17A therapy resolves skin lesions and vascular dysfunction in mouse models of psoriasis
A. Brandl1, R. Schaller1, C. Wohlen1, P. Wenzel1, A. Waisman1, S. Karbach1, B. E. Clausen2; 1Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, 55131 Mainz, Germany, 2Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University Mainz, 55131 Mainz, Germany.

Introduction: Besides skin inflammation, patients with severe psoriasis suffer from an increased risk of cardiovascular mortality. We hypothesized that Interleukin (IL)-17A is a key marker of psoriatic plaque formation and vascular disease. Anti-IL-17A therapy resolved skin lesions and vascular dysfunction in mouse models of psoriasis.

Methods: Constitutive low-level expression of IL-17A by CD11c+ cells resulted in the gradual development of chronic skin lesions, resembling macroscopic, histologic and genetic hallmarks of psoriatic plaques. The onset and incidence but not the severity of cutaneous disease was IL-17A dose dependent. Similarly, vascular dysfunction correlated with peripheral IL-17A levels. Notably, successful anti-IL-17A treatment of psoriatic skin lesions diminished peripheral oxidative stress levels, pro-inflammatory cytokines and vascular inflammation.

Conclusion: These data highlight the pivotal role of IL-17A linking plaque formation and vascular disease in psoriasis. Moreover, neutralization of IL-17A can rescue both the skin and cardiovascular phenotype. Hence, CD11c-IL17A mice represent a valuable tool to investigate the effects of biologics on chronic skin and cardiovascular disease in psoriasis.

P.C1.04.04
PD-1 dependent dysregulation of type-2 innate lymphoid cells in a murine model of obesity
G. Oldenhove1, E. Bouchey1, A. Taquin1, V. Acoby1, L. Bonetti1, B. Ryffel1, M. Le Bert2, K. Englert3, L. Boon1, M. Moser1; 1IBMM, Department of Molecular Biology, Université Libre de Bruxelles, Gosselies, Belgium, 2INEM, UMR7355, Molecular Immunology, University and CNRS, Orléans, France, 3Bioceres, Yelaiean 46, 3584 CM, Utrecht, Netherlands.

Recent observations have clearly established the critical role of type 2 innate lymphoid cells in maintaining the homeostasis of adipose tissues in humans and mice. This cell population promotes browning and limits adiposity directly and indirectly by sustaining a Th2-prone environment enriched in eosiophils and alternatively activated macrophages. Accordingly, the number and function of ILC2s are strongly impaired in obese individuals. In this work, we sought to determine the factor(s) leading to ILC2 destabilization upon high fat feeding. We identified the PD-1/PDL1 pathway as a negative checkpoint of ILC2 homeostasis and function. TNF appears to play a central role, triggering IL-33 dependent PD-1 expression on ILC2s and recruiting/activating PD-L1+ M1 macrophages. PD-1 blockade partially restored the type 2 innate axis, raising a possibility to restore tissue homeostasis.
Human skin is a complex barrier organ with elaborate immune functions to prevent infection by pathogens and reactions against harmless antigens. Numerous immune cells reside in human skin and communicate with each other as well as with the skin tissue cells (e.g. epidermal keratinocytes) in order to mediate appropriate immune responses. We hypothesise that these interactions are crucial to maintain homeostasis within the skin and aim to investigate them. Because murine and human skin differ in structure and cell composition we established a skin-humanized xenograft mouse model to investigate immune mechanisms in vivo. In the model we generate a simplified skin tissue consisting of fibroblasts and keratinocytes only, thus featuring a minimal organotypic skin tissue. This can be reconstituted with different immune cell types, allowing for the study of e.g. T cell-APC, T cell-keratinocyte or T cell-microbe cross-talk. We can successfully follow T cell infiltration of the skin, started to characterize local T cell - APC interactions and found that these lead to improved T cell infiltration of the skin and local T cell proliferation in the presence of a microbial antigen. Further on we will use this model to study the mechanisms of maintenance and recruitment of the numerous skin T cells and their immunological and tissue regenerative function. M.M.K. is supported by a DOC-fellowship from the Austrian Academy of Science. The project was supported by a Debra International Grant awarded to E.M.M. and I.K.G. and a grant from the National Institute of Health (R01AI22772) awarded to D.C.J. and I.K.G.

P.C1.04.06

Hallmark features of atopic skin disease affect keratinocyte-derived exosomal compartment

L. Hovhanissyan1, A. Luczkai2, A. Kabier2, X. Wang1, H. Sheldon1, E. Giannoulaki1, S. Taylor2, G. Ogg2, D. Gutowski-Owsiak1,2

1Institute of Biotechnology UIG, Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Gdansk, Poland; 2MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom, 3State Key Laboratory of Military Stomatomology, Department of Oral Medicine, School of Stomatomology, The Fourth Military Medical University, Xi’an Shaanxi Province, China, 4Cancer Research UK Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom, 5Computational Biology Research Group, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom.

INTRODUCTION: Atopic dermatitis (AD) is a common skin disease combining barrier dysfunction and aberrant epithelial differentiation with inflammation. Recent milestone discoveries highlighted the importance of a keratinocyte protein, filaggrin, linking insufficiency in epidermal barrier with inflammatory state and induction of allergic phenotype resulting in sensitisation to allergens. Exosomes are secreted vesicles acting as messengers between skin cells; they have functional impact on recipient cells by delivery of a protein, lipid or nucleic acid cargo. Published data suggest changes in the secretion of vesicles upon cellular maturation, differentiation and exposure to cytokines. Here, we investigated whether the two hallmarks of allergic skin disease, i.e. keratinocyte differentiation/epidermal barrier dysfunction and inflammatory milieu induce changes in keratinocyte exosomal compartment.

METHODS: We used filaggrin knock-down keratinocytes and allergic mediators (histamine; IL-4/IL-13; IL-17A and IL-22) followed by mRNA profiling and enrichment analysis (Fluidigm tool) to observe changes in keratinocyte exosomal compartment. We isolated exosomes from conditioned keratinocyte medium (by ultracentrifugation) and analysed exosomal output by WB with the use of common exosomal markers.

RESULTS: Expression of genes encoding exosome-enriched proteins was altered in both filaggrin-insufficient keratinocytes and keratinocytes exposed to inflammatory mediators enriched in atopic skin. Western blot analysis confirmed the impact of keratinocyte differentiation state and expression on exosome output. The alterations on the expression level of these proteins has not yet been translated into functional changes.

CONCLUSION: These results suggest changes in inter-cellular communication across skin barrier. These are likely to contribute to pathogenesis of AD. The mechanisms remain largely elusive and warrant further study.

P.C1.04.07

Telomeres of T cells as a marker of type 1 diabetes progression in children

D. Iwaszkiewicz-Grzes1, M. Giwisinski1, A. Wołoszyn-Durkiewicz1, J. Sakowski1, M. Zalińska1, M. Henning1, M. Myśliwiec1, P. Trzonkowski1

1Department of Clinical Immunology and Transplantology, Medical University of Gdansk, Gdansk, Poland, 2Department of Pediatric Diabetology and Endocrinology, Medical University of Gdansk, Gdansk, Poland.

Introduction: Type 1 diabetes (T1D) is a chronic disease caused by autoimmune destruction of beta-cells in pancreatic islets, which is followed by hyperglycaemia and clinical symptoms. These symptoms and environmental factors play an important role in pathogenesis of T1D. Chronic inflammation associated with autoimmune diseases influences the immune system affecting the replication of lymphocytes. It has been observed that in relation to chronic inflammation, immune cells proliferate more intensively, which causes acceleration of cell-aging. Cellular age can be estimated by measuring the telomeres length. In somatic cells, telomeres get shorter with every cell division and as a result, they are the indicator of the cellular senescence.

Methods and Materials: Samples from were collected from 29 patients with newly diagnosed and 25 patients with long-standing type 1 diabetes. CD4+ and CD8+ T cells were isolated from fresh peripheral blood mononuclear cells. We measured telomeric sequences in vertebrate interphase hematopoietic cells. We used MOLT-4 cells as a reference.

Results: We observed changes in inter-cellular communication across skin barrier. These are likely to contribute to pathogenesis of AD. The mechanisms remain largely elusive and warrant further study.

P.C1.04.08

The effect of progesterone on MMP7 and MMP13 expression in mouse model of systemic sclerosis

E. Izyd2, F. Fasahoo2, M. Assarehzadegan1, H. Pooqmohim2, J. Kuhpayehzadeh2, N. Majeelab2

1Immunology department, Iran University of Medical Sciences, Tehran, Iran, 2Iran Islamic Republic of. 3Sclerodema Study group, Firuzgar Hospital,Iran University of Medical sciences., Tehran, Iran, 3Iran Islamic Republic of. 4Immunology department, Iran University of Medical sciences,Iran university medical science, Tehran, Iran, Islamic Republic of.

Background: Gender medicine is a new era of science which focuses on the impact of sex hormones and gender on normal physiology, pathobiology and clinical features of diseases. In our previous study we showed that supra physiological dose of progesterone exacerbate the lung fibrosis in a mouse model of scleroderma. Matrix metalloproteinases are a group of enzymes which play a role in tissue remodeling and fibrosis. Whereas the abnormal expression of MMP2 and MMP9 are indicated in the pathogenesis of systemic sclerosis, fewer discoveries highlighted the importance of a keratinocyte protein, filaggrin, linking insufficiency in epidermal barrier with inflammatory state and induction of allergic phenotype resulting in sensitisation to allergens.

Method: Female mice received progesterone for 28 and 21 days in addition to 28 days bleomycin. On day 29 mice were sacrificed and the expressions of these two enzymes in lung fibrosis of female mice received progesterone for 28 and 21 days in addition to 28 days bleomycin. On day 29 mice were sacrificed and the expressions of these two enzymes in lung fibrosis were measured by WB and PCR.

Results: The expression of MMP7 and MMP13 expression was increased in female mice received progesterone compared to control group. While progesterone cannot reduce the expression of MMP7 and MMP13 in a mouse model of lung fibrosis more investigation on other player of fibrosis are necessary.

Conclusion: Further on we will use this model to study the mechanisms of maintenance and recruitment of the numerous skin T cells and their immunological and tissue regenerative function. M.M.K. is supported by a DOC-fellowship from the Austrian Academy of Science. The project was supported by a Debra International Grant awarded to E.M.M. and I.K.G. and a grant from the National Institute of Health (R01AI22772) awarded to D.C.J. and I.K.G.
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P.C1.04.10
The role of IL-19 in CCl_4-induced liver fibrosis
M. Miški, H. Nokajima, T. Takesuchi, Y. Azuma; Laboratory of Veterinary Pharmacology, Osaka Prefecture University Graduate School of Life and Environmental Science, Izumisano, Japan.

IL-19 is a member of the IL-10 family and is an anti-inflammatory cytokine produced mainly by macrophages. Liver fibrosis results from chronic liver injury-mediated inflammation and activation of hepatic stellate cells. However, the involvement of IL-19 in liver fibrosis is not yet fully understood. We investigated the immunological role of IL-19 in carbon tetrachloride (CCl_4)-induced liver fibrosis model mice. BALB/c genetic background IL-19 knockout (KO) mice and age-matched wild-type (WT) mice were used. Liver fibrosis was induced by CCl_4 injection (2.0 mL of 50% solution/kg, 2 times per week) for 8 weeks. Histological evaluation in the liver was assessed by HE-staining, Azan staining, and α-SMA immunohistochemistry. mRNA expression in the liver was analyzed by quantitative real-time PCR. In CCl_4-induced liver fibrosis, serum analysis revealed that level of ALT was decreased in CCl_4 KO mice compared with WT mice. IL-19 KO mice presented exacerbated fibrosis by the morphometric assessment of the total area positively stained with Azan. Moreover, α-SMA expression was increased in liver sections of IL-19 KO compared with WT mice. Additionally, mRNA expression levels of TGF-β and α-SMA were increased in IL-19 KO mice compared with WT mice. These findings indicate that IL-19 has previously undetermined roles in the progress of fibrosis. Enhancement of IL-19 signaling pathway may present promising therapeutic treatments of liver fibrosis.

P.C1.04.11
Biomarkers in immune response of fungi inoculated mice
A. J. Odebo,1 A. Adenike,1 E. Farombi; 1Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria, 2Department of Botany, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria, 3Faculty of Basic Medical Science, University of Medicine, Ibadan., Ibadan., Nigeria.

Fungi are an increasing public health problem worldwide because they have a great impact on human health. A mouse model was devised to compare the adverse effects provoked by four most abundant fungi isolated from southwest, Nigeria. Sixty BALB/c albinino mice were grouped into nine treatments of six per cage with a control group. The animals were exposed intranasally to the spores of A. flavus, P. chrysogenum, Penicillium citrinum and Penicillium chrysogenum at 2.3 x 10^8 and 3.2 x 10^8/mL for 24 hours. Both dose-response, time-course inflammatory and toxic responses were investigated after a single dose of the microbes. The spores of A. flavus, and P. chrysogenum provoked a very intense acute inflammation indicated by production of increased malondialdehyde, myeloperoxidase, protein in the lungs. The inflammatory cell response in the lungs was more severe and varied with each organism. White blood cells in research the role of both Treg-cells and IL-19 KO mice. Moreover, the infiltration of neutrophils and eosinophils was higher in CCl_4-induced liver fibrosis model mice. All the fungi inoculated resulted in significant (p<0.05) increase in the mononuclear and basophilic cell. P. citrinum significantly increased the eosinophils as A. terreus increased the lymphocytes. The neutrophils content was higher in the A. pennisoloides, P. chrysogenum and P. citrinum inoculated treatments. Both haemoglobin and red blood cell count were significantly increased in the mice inoculated with A. fumigatus, P. chrysogenum and A. flavus compared to control group. The results show that the selected microbes have potential to cause inflammatory and toxic responses after airway exposure in mice.

P.C1.04.12
Cutaneous drug eruptions
L. Pajaziti, S. Sapajni, A. Krasniqi, O. Vogel, A. Pajaziti; University Clinical Center of Kosovo, Prishtina, Kosovo, Republic of.

Drug eruptions are common. Their frequency increases with the increased use of drugs. The intensity of the reactions is different and ranges from mild forms that are more frequent to life-threatening. Clinical manifestations of these reactions are different. The aim of this study is to explore some of the characteristics of the drug reactions in our hospitalized patients: the most common clinical forms of the cutaneous drug eruptions, the identification of the causative drugs, circumstances, and the possible correlation of a drug with a given clinical type. In this study, 128 patients (58 women and 70 males) with cutaneous drug eruption were included.

Six types of reaction were observed: erythema multiforme / Steven-Johnson syndrome, urticaria, exanthematous drug eruption, fixed drug eruption, erythema nodosum, and angioedema. Of them, the dominant pattern was erythema multiforme (34.37%), followed by urticaria (31.25%), and exanthematous drug eruption (19.53%). Nonsteroidal anti-inflammatory drugs and antibiotics were the most common cause of drug reactions, followed by antibiotics (39.06%). A drug may cause some clinical patterns of reaction. Uncontrolled use of nonsteroidal anti-inflammatory drugs increases the likelihood of drug eruptions.

P.C1.04.13
IL-35 maintains regulatory T cells phenotype to suppress diabetic nephropathy
E. Eriksson,1 L. Zuo,1 S. Volh1, M. Mejia-Cordova,1 D. Espes,1 L. Thorvaldson,1 M. Blixt,1 P. Carlsson,1 R. Hansell,1 S. Sandler,1 K. Singh; 1Dept. of Medical Cell Biology, Uppsala University, Uppsala, Sweden, 2Dept. of Medical Cell Biology, Uppsala University, Uppsala, Sweden, 3Uppsala University, Uppsala, Sweden.

Diabetes causes an elevation of the blood glucose level and a long-term hyperglycemia that contributes to kidney damage, i.e. diabetic nephropathy (DN). DN exhibits signs of inflammation and kidney infiltration of mononuclear cells. Regulatory T-cells (Treg-cells) maintain the homeostasis of the immune system, specifically by producing anti-inflammatory cytokines. The goal of this study is to understand the role of IL-35 in the regulation of Treg-cells in DN. Our rationale is based on the fact that IL-35 is a unique cytokine with anti-inflammatory properties. IL-35 levels are significantly increased in the mice inoculated with A. terreus, P. chrysogenum compared to control group. The results show that the selected microbes have potential to cause inflammatory and toxic responses after airway exposure in mice.

P.C1.04.14
The role of interleukin-19 in NASH/NASHi mice model
Y. Ishikoh; K. Hirata, H. Nokajima, T. Tsuchi, Y. Azuma; Laboratory of Veterinary Pharmacology, Osaka Prefecture University Graduate School of Life and Environmental Science, Izumisano, Japan.

IL-19 is a member of the IL-10 family and is an anti-inflammatory cytokine produced mainly by macrophages. Nonalcoholic fatty liver disease (NAFLD) is highly associated with the metabolic syndrome and occurs as a severe common form of the disease, nonalcoholic steatohepatitis (NASH). NASH is diagnosed pathologically by histological evaluation of fibrosis, inflammation, and other features, such as hepatocyte ballooning. However, the involvement of IL-19 in liver inflammation and liver fibrosis is not well understood. We investigated the immunological role of IL-19 in diet-induced NASH/NASHi model mice. IL-19 knockout (KO) mice and wild-type (WT) mice (n=6/group) were treated with high-fat diet (60%cal% fat) with 0.1% methionine and 2% cholesterol for 24 hours. Both dose-response, time-course inflammatory and toxic responses were investigated after a single dose of the microbes. The spores of A. flavus, and P. chrysogenum provoked a very intense acute inflammation indicated by production of increased malondialdehyde, myeloperoxidase, protein in the lungs. The inflammatory cell response in the lungs was more severe and varied with each organism. White blood cells in research the role of both Treg-cells and IL-19 KO mice. Moreover, the infiltration of neutrophils and eosinophils was higher in CCl_4-induced liver fibrosis model mice. All the fungi inoculated resulted in significant (p<0.05) increase in the mononuclear and basophilic cell. P. citrinum significantly increased the eosinophils as A. terreus increased the lymphocytes. The neutrophils content was higher in the A. pennisoloides, P. chrysogenum and P. citrinum inoculated treatments. Both haemoglobin and red blood cell count were significantly increased in the mice inoculated with A. fumigatus, P. chrysogenum and A. flavus compared to control group. The results show that the selected microbes have potential to cause inflammatory and toxic responses after airway exposure in mice.

P.C1.04.15
Continual exit of human skin resident memory CD4 T cells that seed distant tissue sites
M. M. Klinzínk,1 P. A. Morawski,2 H. Hoellbacher,1 T. Dührer,1 S. Motley,2 S. R. Vorkhande,2 M. Rosenblum,2 I. J. Campbell,1 I. K. Gratzi; 1University of Salzburg, Salzburg, Austria, 2Benaroya Research Institute, Seattle, WA, United States, 3University of California, San Francisco, San Francisco, CA, United States.

As a barrier organ the skin harbors immune cells that not only provide protection against a myriad of pathogens but also support tissue homeostasis and repair. A large proportion of these immune cells in skin are resident memory T cells (TRM) with unique skin-tropic signatures that are thought to permanently reside in the tissue and not recirculate. We have identified a novel population of circulating CD4^+CLA^-CD103^+ cells in the blood of healthy humans. Using a multidimensional mass cytometry approach (CyTOF) we found that these cells have a unique skin-tropic phenotype reminiscent of skin TRM. Phenotypic and transcriptional similarities determined by conventional flow cytometry and RNA sequencing further highlighted the close relationship between CD4^+CLA^-CD103^+ TRM and circulating CD4^+CLA^-CD103^+ cells. Their unique cytokine production profile of IL-22 and IL-13, and low production of IL-17A and IFN-γ indicates a function in skin homeostasis and repair. The presence of this unique population in circulation suggests recirculating TRMs, mobilized potentially to seed remote tissue sites.
POSTER PRESENTATIONS

To test this hypothesis, we performed xen-graft experiments, by transferring human full thickness skin onto immunodeficient mice that carried an engineered human skin graft derived from TRM. We found that CLA+CD103+TRM could be mobilized from the skin, enter circulation and seed a distant skin site, while preserving their phenotype. Further, upon transfer of human PBMC we detected CLA+CD103+ cells that had seeded an engineered human skin graft. Thus, we propose that CD4+CLA+CD103+ cells found in the human skin represent a migrating population of skin TRM.

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P.C.1.04.16
Defining the targets of autoimmune disease-associated variation in human Regulatory T cells
Y. Y. Wong;
University of Adelaide, North Adelaide, Australia.

Chromatin structure is known to have a major influence on gene expression by controlling transcription factor (TF) access to binding sites in non-coding regulatory enhancers and promoters. Studies have shown that majority of the autoimmune disease risk associated genetic variations are found in non-coding regions of the genome such as enhancers. Our lab has been working on the functional validation of cell-specific enhancers located at autoimmune disease risk loci, in order to explain how genetic variations at these loci contribute to disease. In view of this, identifying the TFs bound to enhancers and promoters is critical for understanding how these elements work and how disease-associated single nucleotide polymorphisms (SNPs) may influence this activity. Currently, our ability to predict genomic binding sites from sequence alone is limited and functional TF binding sites needs to be experimentally validated. ATAC-seq, which simultaneously probe chromatin structure and importantly transcription factor binding, requires relatively few cells making it amenable to use on rare population and small clinical samples.

P.C.1.04.17
Functional characterization of T lymphocytes in coeliac patients with dermatitis herpetiformis: evidence of cross reactivity between tissue and epidermal transglutaminases
University of Florence, Florence, Italy.

INTRODUCTION: Dermatitis herpetiformis (DH) is considered the cutaneous manifestation of coeliac disease (CD), however why only few coeliac patients develop this disorder is still unknown. Epidermal transglutaminase (TG3) has been recently described as the main autoantigen of DH. Here we propose to study phenotypic features and antigen specific response of T cells towards both tissue (TG2) and epidermal transglutaminases in DH compared to CD patients. METHODS: Mononuclear cells from peripheral blood (PBMC), skin and gut biopsies of 14 DH patients and 8 CD patients were polyclonally expanded and evaluated by flow cytometry for expression of Th1, Th2 and Th17 cells associated markers. Phenotypic analysis in the presence of TG2 or TG3 and antigen specificity of expanded T cells has been evaluated by flow cytometry. RESULTS: Flow-cytometric evaluation showed an increased frequency of TNF-alpha producing T cells in skin of DH patients compared to CD, thus confirming the inflammatory status in the epidermal district. More interesting, antigen specificity assays revealed that a cross reactivity occurs between TG2 and TG3 specific T cells, more in DH than in CD patients. CONCLUSIONS: Our data, even if preliminary, provide possible explanation to the mechanisms leading to DH only in some CD patients. Moreover, based on the finding of the increased production of TNF-alpha at skin level, new possible treatments should be proposed, including biological therapy, being DH very slow to respond to gluten free diet.

P.C.1.04.18
Linking diet, gut immunity and microbiota in the pathogenesis of Type 1 Diabetes
I. Cosorich1, A. De Giorgi1, A. Bolla1, R. Ferrarese1, E. Bosi1, R. Zuppardo1, A. Mariani2, E. Esposito2, M. Falcone2;
1San Raffaele Scientific Institute, Milan, Italy, 2San Raffaele Hospital, Milan, Italy.

Recent data indicate that gut immunity and the mechanisms that regulate effector and regulatory T cell differentiation in the intestine are instrumental to maintain immune tolerance towards self-tissues and to prevent extra-intestinal autoimmune diseases. This observation led to the hypothesis that environmental factors as diet and microbiota modifications, affect the pathogenesis of autoimmune Type 1 Diabetes (T1D). To this aim, we analysed gut mucosal immune cell subsets in tissue samples isolated from the small intestine of T1D patients and healthy controls (HC). A phenotypical analysis of gut mucosal immune cell subsets has been performed. We observed a statistically significant increase of Th2 cells and CD1c+CX3CR1+DCs in the gut of T1D. In order to find if there is a correlative link between diet and Th subsets, we are collecting a 3-days-food record questionnaire from T1D patients. Gut microbiota of brushing material from duodenal was analyzed by 16S rRNA sequencing.

We also investigated if different type of diet can influence autoimmunity in preclinical models of T1D. In particular if an anti-inflammatory diet enriched in fibres and omega3 can reduce gut inflammation and protect NOD mice from T1D. We demonstrated a lower gut permeability in NOD mice fed with omega3 diet, compared to NOD mice fed with control diet. We further aim at elucidating the link of gut immunity alterations and environmental factors that might have a strong impact on T1D.

P.C.1.04.19
Lymph node stromal cells confer location-dependent tolerogenic properties to dendritic cells
J. Pezold1, M. Sente-Pastor1, M. Zou1, C. Wiersch1;
1Heilmanns Centre for Infectious Research, Braunschweig, Germany, 2Biomedical Center, Ludwig-Maximilians University, Munich, Germany.

The balance between regulatory T cells (Tregs) and effector T cells is key for the maintenance of immune homeostasis. To dissect the contribution of lymph node (LN) stromal cells to T cell differentiation and peripheral de novo Treg induction in gut-draining mesenteric lymph nodes (mLNs), LN transplantation experiments have been utilized in a mouse model. Remarkably, the high Treg-inducing capacity of mLNs was retained after transplantation into the popliteal fossa, a non-tolerogenic, skin-draining site, although the hematopoietic compartment was completely replaced after 8 weeks, suggesting mLN stromal cells were stably imprinted with the tolerogenic properties. Additionally, the composition of the autoimmune disease (re) dendritic cell (DCs) and their transcriptional signatures in transplanted mLN closely resemble resDCs from endogenous mLNs. Furthermore, co-cultures of resDCs re-isolated from transplanted mLN with naive CD4+ T cells from D011.10 mice resulted in a high Treg induction, demonstrating that resDCs can get modulated by LN stromal cells after their LN entry, and that mLN stromal cells via modulation of resDCs affect local T cell differentiation and particularly de novo Treg induction. Finally, single-cell RNAseq data from either pLN or mLN CD4+CD24+ stromal cells revealed a to date underappreciated location-dependent heterogeneity. To discern the imprinted tolerogenic properties of stromal cells from different LNs, in vitro experiments of pLN vs mLN stromal cell subsets with precursor DCs (pre-DCs) are currently underway. In conclusion, our recent data demonstrated a cross talk between LN stromal cells and resDCs, which is in turn affecting the generation and/or homeostasis of the Treg compartment.

P.C.1.04.20
Fine characterization of healthy conjunctiva: main findings when comparing IELs and peripheral blood fine lymphocytes subsets
A. Coreli, J. Zarzuela1, C. Martin1, A. Armenta1, R. Reinaos1, S. Rubio1, M. Cano1, A. Valledola1, J. Herreras1;
1Universidad de Valladolid, Valladolid, Spain, 2Centro de Hemoterapia y hemodonación Castilla y León, Valladolid, Spain.

Introduction: As occurs in other mucosal tissues —for example gut, bronchi and nose—, ocular mucosa holds a conjunctiva-associated lymphoid tissue (CALT). It is well known that MALT (Mucosa Associated Lymphoid Tissue) has morphological and functional variations across tissues. Therefore, a thorough analysis of lymphoid populations might render useful information on oral surface conditions. Objectives: The aim of this study is to improve the knowledge of human immune system within the conjunctiva in healthy and different oral surface conditions. Material and methods: Twenty-five healthy volunteers were recruited. Peripheral blood lymphocytes were obtained by venipuncture while intraepithelial lymphocytes (IELs) from eye tissue were obtained by brush cytology. Major and fine subsets were characterized by flow cytometry. Memory, naïve, γδ T cells, CD8+ (Tc, NKT subtypes), CD4+ (Th0, Th1, Th2, Th17, Th1/Th17, Th22 and Treg subsets), B cells (B, and g) and NK cells —regulatory and cytotoxic— subsets were analyzed in both conjunctival mucosa and peripheral blood. Results: Age and sex seemed to determine few differences in some lymphocyte subsets. Th1 cells might be age influenced whereas Th22 might be sex influenced. As expected, no strong correlations between peripheral and conjunctival lymphocytes were found. Conjunctival T cells seemed to be mainly CD8+ and TCRγδ+, while they were only a minor population in peripheral blood. Conclusions: Peripheral CD4+ T cells, NKT, B1, Tregs and regulatory NK cells had higher values in conjunctiva. This suggests specialized functions (including regulatory) in the area.
P.C1.05 Maintenance and local regulation of tissue specific immunity - Part 5

P.C1.05.01 Neutrophil activation by immunoglobulin A exacerbates pathogenesis of inflammatory bowel disease
A. Bos, M. Bögel, R. Mebius, M. van Egmund; VU medical center, Amsterdam, Netherlands.

Immunoglobulin A (IgA) is the most prominent antibody in the mucosa and important for maintaining homeostasis. However, altered IgA repertoires have been found in chronic inflammatory diseases. For instance, patients with inflammatory bowel disease (IBD) have altered IgA against commensal bacteria. Therefore, this study aims to determine the contribution of IgA on IBD pathology.

We used a DSS-induced colitis model to investigate the development of colitis in human IgA x human IgA Fc receptor (hIgA x hFcR) mice. Compared to control mice, hIgA x hFcR mice showed more severe inflammation, reduced body weight and worse survival. Additionally, massive accumulation of intra-intestinal FcβR+ neutrophils was observed. Similarly, inflamed patient biopsies revealed significant infiltration of neutrophils, together with a destroyed epithelial lining. Therefore we assessed the effect of IgA-activated neutrophils on epithelial cell line co-cultures. We observed that IgA-activated neutrophils from IBD patients form neutrophil extracellular traps (NETs) that capture epithelial cells. Importantly, we assessed IBD plasma for the presence of anti-epithelial antibodies and found specifically IgA antibodies recognizing epithelial cell lines. Nevertheless, no enhanced IgG or IgM antibodies were found against epithelial cells within these patients. Thus, we propose that anti-epithelial IgA contributes to IBD pathology by binding to the human epithelial lining, which activates neutrophils and initiates perpetual neutrophil activation, which results in massive tissue damage.

P.C1.05.02 Tissue resident memory cells T cells in the human conjunctiva and immune signatures in human ocular surface diseases
T. Base1, L. Tong2, G. K. Chandy1; 1Ludwigs-Maximilians-Universität, Munich, Germany, 2Singapore Eye Research Institute, Singapore, Singapore, 3Lee Kong Chian School of Medicine, Singapore, Singapore.

Non-recruiting resident memory (T RM) and recirculating T cells mount vigorous immune responses to both self and foreign antigens in barrier tissues like the skin, lung and gastrointestinal tract. Using impression cytology followed by flow cytometry we identified two T RM subsets and four recirculating T subsets in the healthy human ocular surface. In dry eye disease, principal component analysis (PCA) revealed two clusters of patients with distinct T cell signatures. Increased conjunctival central memory and naive T cell counts were observed in patients with dry eye, as compared to healthy controls.

P.C1.05.03 The local microenvironment drives the identity of tissue-resident lymphocytes
S. Christ1, M. Evrard2, D. Newman2, S. L. Park3, J. E. Pier1, F. R. Carbone1, F. Gimona1, A. Kellier1, L. K. Mackay1; 1The Peter Doherty Institute, Melbourne, Australia, 2Singapore Immunology Network (SIgN) Agency for Science, Technology and Research (A*STAR), Singapore, Singapore.

Tissue-resident memory T (T RM) cells are a population of non-recruiting lymphocytes that permanently reside in non-lymphoid organs. Together with other tissue-resident lymphocytes, these cells are critical for controlling tissue infection and cancer, and are implicated in tissue repair and autoimmunity. Although T RM cells possess a common molecular signature distinguishing them from their circulating counterparts, the transcriptional identity of these cells differs vastly between organs. We found that organ-specific gene expression axes are conserved between different resident lymphocytes within a given tissue, partly reflecting differences in local cytokine imprinting. We show that whilst T RM cells in different tissues exhibit differential cytokine and molecular requirements for their development, locally-derived factors cooperate to globally suppress tissue-egress genes in resident cells across all organs. Collectively, our data demonstrate the adaptation of T RM cells to specific tissue microenvironments. Exploiting such commonalities and differences in T RM cell regulation will inform new strategies designed to target these cells in a site-specific manner.

P.C1.05.04 Resident T cells trigger disease-associated tissue responses that stratify clinical outcome in human psoriasis
I. Gallais-Séréal, S. Cheuk, L. Eidsmo; Department of Medicine Solna, Stockholm, Sweden.

Resident T cells provide barrier immunity in murine models of viral infections. In contrast, alteration in functionally distinct subsets of resident T cells is implicated in human focal skin diseases. We recently showed that CD49α marks CD10 T cells poised to cytokine and IFN-γ production, while IL-17 producing T cells form localised disease memories in resolved psoriasis. However, if and how these cells cause human pathology is not known. Here, we investigated the consequences of T cell activation on tissue response patterns. T cell activation induced type-1 interferon tissue responses in explanted skin tissue and psoriasisform, IL-17-related responses were selectively induced in psoriasis-derived skin tissues. Our data indicates that whilst T cell activation can drive clinically relevant tissue responses and stratification of local these responses in resolved psoriasis were correlated to clinical outcome. Finally, our data indicates that microbial interplay with genetically predisposed keratinocytes may shape the local population of resident T cells.

P.C1.05.05 Helicobacter hepatitis as disease driver in a novel CD40-mediated spontaneous colitis-model
V. Friedreich1, C. Barthels1, A. Ogrinc1, D. Garzetti3, I. Forné1, A. Imhof4, T. Brocker1; 1Institute for Immunology, Biomedical Center, Ludwig-Maximilian-University, Munich, Germany, 2Graduate School of Quantitative Biosciences (GQB), Ludwig-Maximilian-University, Munich, Germany, 3Max von Pettenkofer-Institute, Ludwig-Maximilian-University, Munich, Germany, 4Protein Analysis Unit, Biomedical Center, Ludwig-Maximilian-University, Munich, Germany.

The mammalian gastrointestinal tract is shaped by microbiota. To maintain mucosal homeostasis, a balance between appropriate immune responses to invading pathogens and tolerance to food and commensal-derived antigens is essential. Disturbed balances can result in severe inflammatory disorders like Inflammatory Bowel Disease. Dendritic cells (DCs) play a key role in this regulation as they can induce both, immunity and tolerance. To investigate the role of the CD40-CD40 axis in tolerance versus. immunity and the role of DCs therein, we generated a murine model with constitutive CD40-signaling in DC. CD40-signaling leads to migration of CD103+ DCs from the colon lamina propria to draining lymph nodes, followed by DC apoptosis. This loss of CD103+ DCs caused lack of RORγt+Helios induced regulatory T cells and an increase of Th1/Th17 effector cells in the colon, resulting in breakdown of mucosal tolerance and severe colitis.

We used sera from these mice to isolate fcal antigens recognized by mice with helicobacter pylori and studied changes of the microbiota during disease development. We detected Helicobacter pylori-specific antibodies in transgenic mice and could protect them from disease onset by rendering them H. pylori free. Upon H. pylori re-infection of transgenic mice, rapid disease onset was observed. Our data suggest that H. pylori is the disease driver in a CD40-mediated spontaneous colitis-model, allowing us to study T cell specificities and differentiation plasticity during inflammation more in detail.

P.C1.05.07 Canonical and non-canonical functions of tyrosine kinase 2 during liver inflammation
D. Gogova1,2, C. Cassina1, S. Knapp3, A. Puga1, M. Müller1, B. Srab1; 1Institute of Animal Breeding and Genetics, Vienna, Austria, 2Biomodels Austria, University of Veterinary Medicine Vienna, Vienna, Austria, 3CellM (Research Center for Molecular Medicine of the Austrian Academy of Sciences) and Laboratory of Infection Biology, Department of Medicine I, Medical University, Vienna, Austria.

Tyrosine kinase 2 (TK2) belongs to the Janus kinase family of receptor-associated tyrosine kinases and is an integral part of signalling cascades utilized by many cytokines with important immune regulatory activities. Mice deficient for Tyk2 (Tyk2−/−) or expressing enzymatically inactive Tyk2 (Tyk2K923E) are resistant to chronic inflammation and cancer, and are implicated in promoting tissue repair and autoimmunity. Although their disease phenotypes, both in vivo and in vitro, are similar to controls, their development, function, and responses are drastically different from controls.

We found that TK2 and its kinase activity are required to protect from Escherichia coli-induced liver injury, which correlated with diminished systemic and hepatic levels of interleukin-22 (IL-22). IL-22 signals through TK2, produced by innate and adaptive immune cells upon stimulus with various cytokines and growth factors and has been reported to have hepatoprotective effects. Next, we employed the concanavalin A (ConA)-induced acute hepatitis model to further study the role of TK2 in IL-22 production and signalling, as previous studies showed increased disease severity in IL-22−/− mice. We show that IL-22−/− mice have significantly increased liver damage parameters and decreased systemic and hepatic levels of IL-22, whereas TK2−/− mice showed liver damage levels comparable to wild-type mice. Analysis of disease-driving cytokines revealed that hepatic Ifng mRNA expression was almost completely abolished in Tyk2−/− and Tyk2K923E mice, whereas Tnfα expression was specifically reduced in Tyk2K923E animals. Taken together, we show that TK2 protects from infection- and inflammation-induced hepatitis and that TK2K923E is capable to prevent inflammatory TNFα production and ameliorate liver injury. Supported by FWF grants P25642-B22, SFB-F6101 and SFB-F6106.
POSTER PRESENTATIONS

**P.C1.05.08**
A single nucleotide polymorphism in the promoter region of the inhibitory immune receptor SIRL-1 controls its surface expression on mononuclear phagocytes

D. Gollnast1, I. van Capelle2, B. Giovannone3, M. van der Vil4, D. Hijnen1, E. de Jong1, H. Radeke1
1Laboratory of Translational Immunology (UMCU), Utrecht, Netherlands, 2Academic Medical Center, Amsterdam, Netherlands, 3Dermatology and Allergology (UMCU), Utrecht, Netherlands.

Signal inhibitory receptor on leukocytes-1 (SIRL-1) is expressed highly on human blood granulocytes and monocytes and low on circulating myeloid dendritic cells (mDCs) and basophils. SIRL-1 ligation inhibits innate effector mechanisms such as production of Fcε-induced reactive oxygen species in monocytes and neutrophils and NETosis in neutrophils. We found that the single nucleotide polymorphism (SNP) rs6125295C, located in the SIRL-1 promoter region, abrogates SIRL-1 expression in monocytes and dendritic cells, whereas expression levels in neutrophils and eosinophils remain unaffected. Using targeted association analysis, rs6125295C could be associated with the skin inflammatory disease atopic dermatitis. By FACS analysis we found that in healthy skin, SIRL-1 is restricted to very low expression on subpopulations of skin-resident DCs. By RT-PCR analysis of FACS-sorted skin-resident immune cells we identified SIRL-1 mRNA exclusively in dermal mDCs with elevated levels in CD14+ IV- like macrophages. In individuals that carry the rs6125295C allele, SIRL-1 transcripts were undetectable in these dermal cell types, indicative of similar transcriptional regulation of SIRL-1 in skin-resident DCs as in blood-resident monocytes and mDCs. In contrast to skin, high expression of SIRL-1 was detected on 30-50% of tissue-resident immune cells in healthy lung tissue samples derived from tumor-removal surgery. SIRL-1 was expressed on interstitial macrophages, monocytes, and mDC subsets.

These results suggest a role of SIRL-1 in immune regulation of mononuclear cells, including monocytes, mDCs and macrophages. Immune inhibitory functions of SIRL-1 may be important to circumvent the manifestation of inflammatory diseases in human barrier tissues and may be of particular importance in lung.

**P.C1.05.09**
Massive and organized B-cell infiltrates in the aorta of LV-GCA patients

J. C. Graver1, M. Sandovici1, E. A. Hoackle1, A. M. Boots1, E. Brouwer1
1University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Giant cell arteritis (GCA) is the most common type of systemic vasculitis and can be classified into cranial (c-GCA) and large-vessel (LV)-GCA. GCA is postulated to be T-cell-mediated and in temporal artery infiltrates, T-cells clearly outnumber B-cells. However, our report on a disturbed homeostasis of B-cells in newly diagnosed GCA patients shows evidence for a role of B-cells. So far, the role of B-cells in GCA is underexplored and the presence of B-cells in the vessel wall of LV-GCA patients is unknown. Therefore, this study assessed the presence of B-cells in the aorta of LV-GCA patients.

Aorta tissue samples of 9 histologically-proven LV-GCA patients who underwent surgery due to an aortic aneurysm were studied by immunohistochemistry. Staining was performed with antibodies against CD20 (B-cells), CD21 (follicular dendritic cells (FDC)), bcl2 (germinal centers), and CD138 (plasma cells). None of the patients received immunosuppressive treatment at the time of surgery. Aorta tissue from age- and sex-matched atherosclerosis patients with an aneurysm were included as controls.

Aorta tissues of LV-GCA patients showed massive infiltration of B-cells, mainly in the adventitia. In contrast to the temporal artery, B-cells outnumbered T-cells in the aorta. B-cells organized into artery tertiary lymphoid organs; there was co-localization of B- and T-cells, FDCs, germinal centers and plasma cell niches.

Aorta tissues from patients with histologically-proven LV-GCA showed massive and organized B-cell infiltrates in the adventitia. The mere presence of B-cells at the site of inflammation promotes further investigation into the role of B-cells in GCA.

**P.C1.05.10**
Tryptophan metabolism in inflammatory bowel disease - Distribution and regulation of enzymes, metabolites and target structures in a multidimensional model

M. Huhn1, M. Herrera San Juan2, R. Boll3, J. Pelischifer4, P. Weiler, H. Radeke5
1pharmazeum frankfurt, Frankfurt am Main, Germany, 2Institute for Instrumental Analysis and Bioanalytic, Mannheim, Germany.

The immune pathogenesis of inflammatory bowel diseases leads to a perpetuating mucosal inflammation based on an imbalance of pro- and anti-inflammatory cytokines. While tryptophan (TRP) and kynurenine have been examined in detail, neither downstream enzymes nor other cells involved in chronic inflammation have been studied in detail. Therefore, we sought to complete the knowledge of the enzyme chain in all cell types involved in chronic inflammation. We performed a multidimensional metabolome TRP analysis in primary colon cell lines, cancer cell lines and immune cell lines of healthy donors by LC-MS/MS and r-te-QPCR. Cells of the innate immune system, especially monocytes, dendritic cells and macrophages, were proven to be the main producers of TRP metabolites. In addition, mRNA of indoleamine 2,3-dioxygenase 1 (IDO1) was expressed in cytotoxic CD8+ T-cells and B-cells. T-cells revealed no constitutive expression of IDO1 and IDO2 mRNA. Tryptophan 2,3-dioxygenase (TDO) and kynurenine 3-monooxygenase (KMO) mRNA were detected in cancer cell lines CaCo-2 and DLD-1, but not in primary colon cell line Caco841 CoN. Furthermore, mRNA expression of G protein-coupled receptor (GPR35) was shown to be increased in CaCo-2 and DLD-1. However, only low expression levels of GPR53 mRNA were detected in Caco841 CoN. Our findings reveal an altered TRP metabolism, with an increased activity caused by inflammatory conditions. Taken together, we identified IDO, KMO and GPR53 as potential biomarkers for colorectal cancer.

These results point to a major role in the development and progression of chronic diseases like cancer. Funded by the Arbeitgemeinschaft industrieller-Forschungsvereinigungen 2.

**P.C1.05.11**
A trauma and victimization history associate with Immune barrier dysregulation in women

1CDC, Atlanta, United States, 2Emory University, Atlanta, United States, 3The University of Arizona, Phoenix, United States, 4The University of Arizona, Phoenix, United States.

Background: A growing body of literature suggests women who have experienced psychological traumas such as violence victimization, are more vulnerable to sexually transmitted infections (STIs). Thus, the susceptibility to infections with sexual biological a impact on STI risk through long-lasting immune changes at sites of STI exposure has been suggested. Therefore, we sought to complete the knowledge of the enzyme chain in all cell types involved in chronic inflammation. We performed a multidimensional metabolome TRP analysis in primary colon cell lines, cancer cell lines and immune cell lines of healthy donors by LC-MS/MS and r-te-QPCR. Cells of the innate immune system, especially monocytes, dendritic cells and macrophages, were proven to be the main producers of TRP metabolites. In addition, mRNA of indoleamine 2,3-dioxygenase 1 (IDO1) was expressed in cytotoxic CD8+ T-cells and B-cells. T-cells revealed no constitutive expression of IDO1 and IDO2 mRNA. Tryptophan 2,3-dioxygenase (TDO) and kynurenine 3-monooxygenase (KMO) mRNA were detected in cancer cell lines CaCo-2 and DLD-1, but not in primary colon cell line Caco841 CoN. Furthermore, mRNA expression of G protein-coupled receptor (GPR35) was shown to be increased in CaCo-2 and DLD-1. However, only low expression levels of GPR53 mRNA were detected in Caco841 CoN. Our findings reveal an altered TRP metabolism, with an increased activity caused by inflammatory conditions. Taken together, we identified IDO, KMO and GPR53 as potential biomarkers for colorectal cancer.

These results point to a major role in the development and progression of chronic diseases like cancer. Funded by the Arbeitgemeinschaft industrieller-Forschungsvereinigungen 2.

**P.C1.05.12**
Understanding the crosstalk between immune and epithelial cells in the development of the “TTP-deficiency” syndrome

C. Le1, B. De Toeye2, A. Assabban3, H. Shehade4, E. A. Haacke5, A. M. Boots6, E. Brouwer7
1Institut für Medizinische Immunologie, Gosselies, Belgium, 2Laboratory of Molecular Biology of the Gene, Institut de Biologie et de Médecine Moléculaire, Gosselies, Belgium, 3Duke University Medical Center, Durham, United States.

Introduction: Interactions between epithelial barriers and environmental stimuli are essential for a functional immunity, to maintain homeostasis and avoid tissue damage. Tristetraprolin (TTP, encoded by Zfp36) is expressed on interstitial macrophages, monocytes, and mDC subsets; however, mice harboring conditional TTP deletion in myeloid cells (LysM-Cre; Zfp36fl/fl) do not develop any spontaneous pathology. In sharp contrast, TTP deletion in keratinocytes (K14Cre; Zfp36fl/fl) mice) and immune cells and the Zfp36 family in the gut of TTPKO mice. 4) There was no overt intestinal inflammation histologically but mRNA expression of cytokines and inflammatory markers was increased in the ileum. We thus hypothesize that absence of TTP might also activate regulatory mechanisms controlling this subclinical inflammation. Indeed we observed higher levels of IL-22 and increased Tregs in the lamina propria. Perspectives. To further study the role of TTP in epithelial (using VillinCre Zfp36fl/fl) mice and immune cells and the role of the gut microbiota in the development of the TTP deficiency syndrome.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

Poster Presentations

P.C1.05.11
ASCs in CD4+ T cells intrinsically limit their proliferation capacity and is required to maintain intestinal homeostasis
H. Jovanmann Khameneh1, A. Mortelloro2,3
1Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (*ASTAR), Singapore, Singapore, 2San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy.

The apoptosis-associated speck-like protein containing a CARD (ASC or Pycard) plays a pivotal role in the assembly and activation of the inflammasome complex. Inflammasome activation is essential for caspase-1 activation in response to diverse danger and pathogen-associated signals, which leads to proteolytic cleavage and release of the pro-inflammatory cytokines IL-1beta and IL-18. ASC has been shown to play important roles in the context of inflammation, cell death, and tumorigenesis. While ASC has been broadly implicated in inflammasome activation in myeloid cells, such as macrophages and neutrophils, little is known about its contribution in lymphocyte biology. Here, we found that Asc is expressed in naïve T cells and its loss resulted in increased proliferation in vitro and in vivo, indicating that ASC intrinsically fine-tunes proliferative capacity of CD4+ T cells. Using a mouse model of induced chronic colitis, we found that Asc expression in CD4+ T cells intrinsically suppresses their proliferative capacity, facilitating the maintenance of gut homeostasis by the mucosal adaptive immune system. Further analysis of Asc+ CD4+ T cells revealed a stronger TCR signaling and an altered metabolic profile in these cells compared to wild-type CD4+ T cells. In conclusion, ASC in CD4+ T cells has crucial non-inflammasome functions to modulate T-cell biology and maintenance of mucosal immune homeostasis in the gut.

P.C1.05.14
The co-inhibitory molecule PD-L1 contributes to regulated inflammation in murine crescentic glomerulonephritis
K. Neumann1, A. Ochs1,3, A. Och1, F. Tacke1, H. Paust1, U. Panzer1, A. Teg2
1University Medical Center Hamburg-Eppendorf, Hamburg, Germany, 2RWTH-University Hospital Aachen, Aachen, Germany.

Introduction: Crescentic glomerulonephritis is a severe glomerular disease mediated by inappropriately regulated cellular and humoral immune responses subsequently leading to development of end-stage renal failure. Previously, we demonstrated a crucial role for regulatory T cells (Treg) in controlling the inflammatory Th1 immune response during nephrotic nephritis ( NTN), the murine model of crescentic glomerulonephritis. Here, we aim at investigating the role of the co-inhibitory molecule PD-L1 in Treg-mediated protection from NTN.

Methods: NTN was induced by i.p. injection of nephrotic serum. Analysis was done eight days later. Kidney damage was analyzed by quantification of crescent formation and determination of albumin-to-creatinine ratio. Tregs from nephritic PD-L1+ and WT mice were transferred into Rag1-/- mice one day before NTN induction. Cytokine expression was analyzed by quantitative RT-PCR and flow cytometry.

Results: We demonstrated that Foxp3+ Tregs expressing PD-L1 infiltrate the kidney during NTN. In nephritic PD-L1-/- mice, the frequency of renal Tregs was increased compared to WT mice. However, PD-L1+/- mice developed more severe NTN associated with a strongly elevated renal Th1 immune response. The same findings were shown after blockade of PD-L1 in WT mice. Moreover, neutralization of IFNγ in PD-L1-/- mice ameliorated NTN. Interestingly, lack of PD-L1 profoundly altered the gene expression profile of Tregs in kidney homeostasis and inflammation. Functionally, Tregs from nephritic PD-L1-/- mice had impaired suppressive capacity in vitro and did not protect from NTN in vivo.

Conclusion: PD-L1 displays a protective role in NTN, which is related to Treg-mediated suppression of the Th1 immune response.

P.C1.05.15
MicroRNAs as a regulator of development and progression in inflammatory bowel diseases among the Polish population
A. Surwielocka-Postawska1, E. Zakścielnia1, M. Zagóza1, M. Durlik1
1Morskie Okrąglak Medical Research Centre of the Polish Academy of Sciences, Warsaw, Poland, 2Department of Gastroenterological Surgery and Transplantation, Warsaw, Poland.

Introduction: MicroRNAs (miRNAs) have been studied for their role in the development of various immune diseases. MiRNAs are small non-coding RNAs that are able to modulate gene expression by binding to the 3’ UTR of target mRNAs. They can play a role in the development of various immune diseases, including inflammatory bowel disease (IBD). The aim of this study was to investigate the expression of miRNAs in serum samples from patients with IBD and healthy controls.

Methods: We used quantitative real-time RT-PCR to measure the expression of selected miRNAs in serum samples from 60 IBD patients and 50 healthy controls. The expression patterns of the circulating miRNA in serum were quantitatively assayed using reverse transcription and real-time RT-PCR.

Results: We detected significantly up-regulated expression of miR-21 in a serum isolated from IBD patients. Genotype-phenotype correlation analysis revealed that miR-21 expression was associated with severe form of the disease.

Conclusion: In conclusion, miR-21 may be a potential biomarker for the diagnosis and prognosis of IBD.

P.C1.05.16
Gammadopathy, immunodeficiency and autoimmunity: when the immune system turns against us
P. E. Walo Delgado, P. Lapuente-Suanzes, I. Nieto-Gañan, A. Carrasco-Sayalero
Servicio de Immunología. Hospital Universitario Ramón y Cajal, Madrid, Spain.

Introduction. The immune system helps to keep homeostasis. It is capable of recognizing, controlling and eliminating pathogens as well as neoplastic cells. When a dysfunction of the immune system occurs, it can lead to the development of diseases. One of the most common diseases caused by a dysfunction of the immune system is autoimmunity. Autoimmunity is a condition in which the immune system attacks the body’s own tissues. This can lead to a variety of diseases, including rheumatoid arthritis, lupus, multiple sclerosis, and type 1 diabetes.

Conclusion. Despite not being able to find a clear causal correlation between the episodes of autoimmune encephalitis and the several other immunological alterations shown by the patient, it could be hypothesized that the presence of autoimmunity, as well as hypogammaglobulinemia in this case – causing increased susceptibility to infections - is found in the context of bicalonal gammopathy, thus constituting a well-known, classical association between autoimmunity and immunodeficiency.

P.C1.05.17
Characterizing intraepithelial lymphocytes in human bile ducts
C. L. Zimmer1,2, E. Van Seth1,2, O. Strauss1, L. Berglin1,2, U. Arnelo1,2, A. Bergquist1,2, A. Mortelloro3
1Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden, 2Department of Hepatology, Karolinska University Hospital, Stockholm, Sweden, 3Department of Gastroenterology and Rheumatology, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

The bile duct is a mucosal barrier tissue connecting the liver with the small intestine. Intraepithelial lymphocytes (IELs) typically reside in epithelial layers of barrier tissues and are thought to play a role in the immunopathogenesis of diseases in bile ducts, such as primary sclerosing cholangitis (PSC), a chronic inflammatory disease with unknown etiology. However, the composition and function of immune cells localizing to bile ducts remain unexplored. Using endoscopy, we obtained brush-samples from the bile duct mucosal surface and carried out an extensive flow cytometric analysis of the biliary immune system. Our results revealed major differences in immune cell composition in bile ducts as compared to peripheral blood. This included the presence of a sizeable population of CD16+CD56+ IELs that was verified using immunofluorescent microscopy. These IELs had a TCRβ+CD3αβ effector memory T cell phenotype, presented with a gut and liver homing chemokine receptor profile, and were highly functional. Taken together, the characterization of the biliary immune system increases our understanding of immune responses at this previously unexplored site and sheds new light on the immunopathogenesis of PSC.
POSTER PRESENTATIONS

P.C1.05.18 Dynamic transcriptional and epigenetic response of CDB + intraepithelial lymphocytes (IELs) to proinflammatory cytokines
M. M. Zorro Manrique1, R. Asquire-Gamboa1, T. Nayouz1, C. Ciszekwi2, C. Wijmenga3, S. Witthoff4, B. Jabl5, Y. Li5, I. Jonkers1
1UMCG, Groningen, Netherlands, 2University of Chicago, Chicago, United States.
Cytokine deregulation contributes to the development of autoimmune diseases (AIDs) by eliciting the activation of immune cells, including cytokitic CDB T cells. These cells are located in mucosal and intestinal tissues, where they detect and destroy pathogens, but are also known to damage the intestinal barrier under disease conditions. Limited recovery from intestinal biopsies has hampered study of CDB Intra Epithelial lymphocytes (IELs). To elucidate the molecular pathways involved in activation of these cells by AID-related cytokines, we investigated the dynamic transcriptional and epigenetic changes and cytokine production of CDB IELs derived from the intestine of patients in response to IL-15, IL-21 or IFNβ at different time points (0h, 30min, 3h, 4h, and 24h).

Our results show unique gene expression profiles for each cytokine. IL-21 promoted moderate gene expression changes (838 differentially expressed genes (DEGs) after 3h), most related to immune pathways. IL-15 and IFNβ provoked strong activation of IELs. IFNβ induced a robust interferon immune response mediated by STAT1, followed by a drastic increase in gene expression of cell cycle genes. IL-21 induced immune- and RNA-processing genes, likely mediated by AP-1 and EGR1, which were mostly restored to resting state after 24h. Although the secretion of cytokines did not follow the patterns of expression of their encoding genes, the transcriptional profiles were mirrored by changes in H3K27ac profiles at genes and enhancers that regulate DEGs.

In conclusion, these cytokite IELs show great plasticity in both genetic and transcriptional profiles in response to the inflammatory milieu.

P.C1.05.19 Mature naive B cells accumulate in interstitial space of term placenta and positively associate with specific chemokines
A. Lunde1, M. Solder2, L. Gorchi3, S. Gid1f4, E. Tabbod5, H. Kaper6
1Department of Rheumatology and Inflammation Research, Sahlgrenska Academy, Gothenburg, Sweden, 2Dept of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, 3Dept of women’s and children's health, Karolinska Institutet, Stockholm, Sweden.
Introduction: Total B cell numbers in the circulation decrease during late pregnancy compared to post-partum and to non-pregnant controls, but the underlying mechanisms for this is unknown. The aim of this study was to examine if the proportions of total B cells and B cell subsets at different maturational stages differ in peripheral blood (PB) compared to placental interstitial blood (IBV) at delivery. Methods: From 23 paired samples of PB and IBV following uncomplicated full term pregnancies, total B cells as well as transitional, mature/naive and memory B cells were identified by flow cytometry. Chemokine levels in blood were analyzed using a luminescy assay. Results: We found that the proportions of total B cells, as well as the fraction of mature/naive B cells, were significantly higher in IBV relative to PB. In contrast, the proportions of immature transitional B cells and memory B cells were higher in PB compared to IBV. Multivariate factor analysis demonstrated that a specific profile of chemokines in IBV, including CCL20, positively associated with higher proportions of mature/naive B cells in the interstitial space. Although all B cells expressed CCR6, the corresponding receptors for CCL20, mature/naive B cells expressed the highest levels of this receptor. Conclusion: these results suggest that B cells, and mature/naive B cells in particular, homed to the placenta in response to certain chemokines produced by this tissue during late pregnancy.

P.C1.05.20 Effects of terminating treatment with ADSC-derived exosomes on obesity
Q. Wang1, H. Zhao2
1School of Basic Medical Sciences, Shandong University, Jinan, China. 2Department of Obstetrics and Gynecology, Shandong University, Jinan, China.
We have recently reported that exosomes from adipose-derived stem cells (ADSCs) attenuate adipose inflammation, insulin resistance or even obesity development in mice fed on high-fat diet. ADSC-derived exosomes drive the polarization of macrophages into anti-inflammatory M2 phenotypes through transactivation of arginase 1, which further mediate binging of white adipose tissue. To optimize the protocol for exosome treatment, the administration of ADSC-derived exosomes was ended in high-fat diet-fed mice that received 6-8 weeks of exosome injection, the adipose tissue inflammation and insulin resistance were evaluated. After 6-8 weeks of exosome treatment, the mice showed significant improvement on glucose tolerance; while 3 weeks after terminating treatment, ADSC-derived exosomes showed no more protection against insulin resistance in obese mice. Four weeks after terminating treatment with exosomes, the mRNA level of arginase 1 in splanchnic vascular fraction from epidydimal fat pad showed no significant elevation despite an increasing trend, while the mRNA level of TNF-α was still significantly decreased; additionally, no obvious decreases in fat mass were observed. These findings suggest that termination of treatment partially reduces the beneficial effects of ADSC-derived exosomes on obesity-associated adipose tissue inflammation and metabolic disorders, which need to be considered during the exosome treatment for obesity. This study is supported by National Natural Science Foundation of China 81471065, 81770838 and Shandong Major Research Program 2016GSF201005.

P.C1.05.21 Tipping the scale - how a shift in the presentation of self-antigen can prime for autoimmunity
J. Petersen1, J. D. Ooi2, H. Reid3, J. Jessen4, R. Kitching5
1Monash University, Clayton, Australia, 2Centre for Inflammatory Diseases, Clayton, Australia, 3Department of Nephrology, Clayton, Australia.
The T cell repertoire of each individual is shaped to finely balance the recognition of foreign antigen against potential autoimmunity. This balance is determined in the thymus, where the development of mature T cells is closely monitored to ensure that self-reactive T cells are selectively deleted. Limited recovery from the thymus is associated with autoimmunity. However, the mechanisms that drive this deletion of self-reactive T cells are largely unknown. Here we present evidence that the thymus can tolerate self-reactive T cells in the presence of other Intra Epithelial Lymphocytes (IELs). To elucidate the molecular pathways involved in activation of these cells by AID-related cytokines, we investigated the dynamic transcriptional and epigenetic changes and cytokine production of CDB IELs derived from the intestine of patients in response to IL-15, IL-21 or IFNβ at different time points (0h, 30min, 3h, 4h, and 24h).

Our results show unique gene expression profiles for each cytokine. IL-21 promoted moderate gene expression changes (838 differentially expressed genes (DEGs) after 3h), most related to immune pathways. IL-15 and IFNβ provoked strong activation of IELs. IFNβ induced a robust interferon immune response mediated by STAT1, followed by a drastic increase in gene expression of cell cycle genes. IL-21 induced immune- and RNA-processing genes, likely mediated by AP-1 and EGR1, which were mostly restored to resting state after 24h. Although the secretion of cytokines did not follow the patterns of expression of their encoding genes, the transcriptional profiles were mirrored by changes in H3K27ac profiles at genes and enhancers that regulate DEGs.

In conclusion, these cytokite IELs show great plasticity in both genetic and transcriptional profiles in response to the inflammatory milieu.

P.C1.06.06 Maintenance and local regulation of tissue specific immunity - Part 6
P. Arodi1, J. Horváth2, E. Kemecsei3, Z. Jakus4
1Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary.
Contact hypersensitivity (CHS) reaction, the mouse model of human allergic contact dermatitis, can be induced by repeated exposure to contact allergens. The lymphatic system is a critical player in the regulation of the immune responses in infectious diseases in the skin but the possible role of lymphatics in the development of allergic contact dermatitis remains unclear.

In our studies Figk R1 mice carrying a germline point mutant kinase dead Vegfr3 allele were used. CHS was initiated by the exposure of the skin to TNCB (2,4,6-trinitrochlorobenzene) followed by a second treatment. The disease progression was monitored by the measurement of ear thickness. The ears were collected for paraffin-based histology followed by H&E staining and immunostaining by lymphatic and immune cell markers.

We found the complete lack of lymphatics in the skin including the ear of Figk R1 mice, while the lymphatic structures were present in the lung and small intestine. We characterized the development of CHS in Figk R1 and Figk R1 mice and our experiments have revealed reduced inflammation in the ear of the Figk R1 mice. CHS development induced dynamic changes in lymphatic morphology and resulted in unexpected lymphatic growth in Figk R1 ears.

Our results revealed that dynamic changes of lymphatic morphology occur in CHS, and the inflammation is reduced in the Figk R1 mice lacking lymphatics in the ears. They also suggest that distinct mechanisms regulate the development and inflammatory lymphangiogenic program. Our findings define novel aspect of the interactions between the immune and lymphatic in allergic diseases.

P.C1.06.06 Role of efficiencies of peptidylarginine deiminase (PAD) 2 and 4 in targeting site distribution in fibrogenic, alpha-enolase and histone H3 for anti-citrullinated protein antibodies
D. Dompardo1, M. Bawadekar2, I. Senol3, A. Stensballe4, M. A. Shelef5, C. H. Nielsen6
1Institute for Inflammation Research, Center for Rheumatology and Spine Diseases, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, 2Department of Medicine, University of Wisconsin, Madison, 3Institute of Rheumatology and Department of Rheumatology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic, 4Department of Health Science and Technology, Aalborg University, Aalborg, Denmark, 5William S. Middleton Memorial Veterans Hospital, Madison, United States.
Introduction: Peptidylarginine deiminase 2 (PAD2) and PAD4 are expressed in the synovium of rheumatoid arthritis (RA) patients and catalyze citrullination of arginine residues in proteins targeted by anti-citrullinated protein antibodies (ACPAs). Little is known about the relative importance of PAD2 and PAD4 in generating citrullinated self-antigens. Here we investigate the ability of PAD2 and PAD4 to generate citrullinated targets of ACPAs in four human proteins.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 317
Materials and Methods: Synovial fluid (SF) and plasma were collected from 42 RA patients. Human fibroblagen, human alpha-enoalase (ENO1), human histone H3, and human serum albumin (HSA) were citrullinated by in vitro Pad2 and Pad4. The total degree of citrullination was determined using the anti-modified citrulline approach. Antibody binding to native and citrullinated proteins was measured by ELISA.

Results: ACPAs within pooled SF from multiple RA patients reacted equally well with Pad2- and Pad4-citrullinated fibroblagen, at any Pad and SF concentration used. Accordingly, ACPAs from most individual patient SF and plasma samples bound equally well to Pad2- and Pad4-citrullinated fibroblagen and ENO1. Native ENO1 was also targeted substantially by ACPAs. When histone H3 was used as target, Pad2 was generally superior in generating epitopes recognized by ACPAs. Despite adequate citrullination, no binding to citrullinated HSA was observed. Conclusion: In most patients, Pad2 and Pad4 are equally efficient in generating citrullinated target sites in fibroblagen and ENO1. The binding of autoantibodies to histone H3 was generally higher after citrullination with Pad4 than with Pad2. Citrullinated HSA is not a target for ACPAs.

P.C1.06.03 Autoantibodies against the basal cell layer recognize keratin 14 and keratin 5 in patients with hepatitis delta virus

J. Delgado de la Pava, C. Garcia Miralles;
Parc Taulí Hospital Universitari, Institut d’Investigació i Innovació Parc Taulí, Universitat Autònoma de Barcelona, Sabadell, Spain.

Introduction: Hepatitis delta is the most severe viral hepatitis leading to hepatic decompensation and hepatocarcinoma rapidly. Zauli D et al. in 1984 described antibodies to the basal cell layer (BCL) in patients with hepatitis delta virus (HDV). To date, anti-BCL antibodies have not been identified. Immunohistochemistry (IHC) on sections of monkey esophagus were realized. Antigen characterization was performed with human and rat epidermal extracts. To confirm the results a capture ELISA has been designed with monoclonal antibodies against recombinant keratin 14 (K14) and keratin 5 (K5).

Results: 49 of the 176 HDV samples (27.8%) have anti-BCL antibodies by IIF and 1 control sample (3.3%). 1 dimension immunoblot on human and rat extracts reveals the presence of the 51 kDa band which, upon sequencing, gave specific identifications for keratins 1, 5, 9, 10, 14 and 16. Only keratins 5 and 14 are present in epidermis BCL, then we design a capture ELISA for K14 and K5 to confirm these antigenic specificities. 114 HDV sera (65.5%) were positive for K14 and 112 HDV sera (64.4%) were positive for K5 and 2 (6.7%) and (3.3%) control sera were positive for K14 and K5 respectively.

Conclusion: The present study has identified K14 and K5 as main target antigens that recognize autoantibodies against BCL. This identification has been confirmed through a capture ELISA with recombinant K14 and K5.

P.C1.06.04 Extraordinary antiphospholipid antibodies in seronegative antiphospholipid syndrome of central nervous system with small vessel brain lesions

1Hospital Universitario Son Espases, Palma de Mallorca, Spain, 2Institut d’Investigación Sanitaria Illes Balears (IdiSba), Palma de Mallorca, Spain, 3Hospital Universitario Miguel Servet, Zaragoza, Spain, 4Hospital Fundación Alcorcón, Madrid, Spain.

Background. Seronegative Antiphospholipid Syndrome (SNAPS) refers to patients with clinical profile suggestive of APS but persistently negative for antiphospholipid antibodies (aPL) included in APS classification criteria: anticardiolipin (ACA), anti-beta2Glycoprotein I (B2GPI) (both IgG or IgM), and lupus anticoagulant.

Patients and methods. We studied 65 patients with small vessel brain lesions (SVBL), MRI and clinical manifestations compatible with APS and aPL negative for aPL included in APS criteria. We also tested 24 autoimmune controls. We performed: 1-ELISA assays for B2GPI and ACA (IgA); anti- phosphodiesterase/prothrombin (PS/PT); phosphatidylcholine, prothrombin (PT) (IgG, IgM) and anti- annexin V (IgG).

2-Chromoluminiscence assay for antibodies to Domain of 1B1 of B2GPI.

Results. We found 13 patients positive for extra-criteria aPL: 2 (3.1%) for B2GPI IgA; 4 (6.1%) for PT IgG, 1 of them was low positive; 4 (6.1%) for PT IgM, 2 were low positive for PS/PT IgM (3.1%) and 1 was low positive for PT IgG and PS/PT IgM (1.5%). Only 3 controls were positive: 1 for PS/PT IgM, 1 for PT IgG and 1 for annexin V all of them positive.

Excluding low positive results, we detected 9 patients and 0 controls positive.

Conclusions. The presence of Abs to B2GPI (IgA) and PT (IgG and IgM) allowed us to identify as aPL-positive 13.8% of seronegative SVBL patients presenting MRI and clinical findings compatible with APS. Follow-up of these patients and additional studies will confirm our results.

This work was funded by a grant from the Spanish Society of Internal Medicine (ref: SAFSN-SNCpv).

P.C1.06.05 Pro-inflammatory histidyl-tRNA synthetase-specific CD4+ T-cells are enriched in the lung of patients with inflammatory myopathies and antisyntetase syndrome

1Immune Institute, Karolinska University Hospital, Stockholm, Sweden, 2Center for Molecular Medicine, Stockholm, Sweden, 3Benaroya Research Institute at Virginia Mason, Seattle, Washington, United States, 4Institute of Clinical Research, Karolinska Institute, Stockholm, Sweden, 5Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden, 6Department of Medical Sciences, Karolinska Institute, Stockholm, Sweden, 7Department of Respiratory Medicine and Allergy, Karolinska University Hospital, Stockholm, Sweden, 8Department of Respiratory Medicine, Karolinska University Hospital, Stockholm, Sweden, 9Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden, 10Respiratory Medicine Unit, Department of Medicine, Karolinska University Hospital, Stockholm, Sweden.

Objectives: To investigate immunity against histidyl-tRNA synthetase (HisRS) in blood and lungs of idiopathic inflammatory myopathy (IIM)/anti-synthetase syndrome (ASS) patients.

Methods: Bronchoalveolar lavage fluid (BALF) cells, and peripheral blood mononuclear cells (PBMCs) from IIM/ASS patients (n=24) were stimulated with full-length HisRS-protein (HisRS) and with HisRS peptide. T-cells are enriched in the lung of patients with inflammatory myopathies and antisynthetase syndrome.

Results. We studied 65 patients with small vessel brain lesions (SVBL), MRI and clinical manifestations compatible with APS and aPL negative for aPL included in APS criteria. We also tested 24 autoimmune controls. We performed: 1-ELISA assays for B2GPI and ACA (IgA); anti- phosphodiesterase/prothrombin (PS/PT); phosphatidylcholine, prothrombin (PT) (IgG, IgM) and anti- annexin V (IgG).

2-Chromoluminiscence assay for antibodies to Domain of 1B1 of B2GPI.

Results. We found 13 patients positive for extra-criteria aPL: 2 (3.1%) for B2GPI IgA; 4 (6.1%) for PT IgG, 1 of them was low positive; 4 (6.1%) for PT IgM, 2 were low positive for PS/PT IgM (3.1%) and 1 was low positive for PT IgG and PS/PT IgM (1.5%). Only 3 controls were positive: 1 for PS/PT IgM, 1 for PT IgG and 1 for annexin V all of them positive.

Excluding low positive results, we detected 9 patients and 0 controls positive.

Conclusions. The presence of Abs to B2GPI (IgA) and PT (IgG and IgM) allowed us to identify as aPL-positive 13.8% of seronegative SVBL patients presenting MRI and clinical findings compatible with APS. Follow-up of these patients and additional studies will confirm our results.

This work was funded by an grant from the Spanish Society of Internal Medicine (ref: SAFSN-SNCpv).

P.C1.06.06 Clinical relevance of the new auto-antibodies in dermatomyositis/polymyositis

1UGC Hematology, Immunology and Genetics. Hospital Puerta del Mar, Cádiz, Spain, 2UGC Dermatology. Hospital Puerta del Mar, Cádiz, Spain.

Introduction: Dermatomyositis and polymyositis (PM/DM) are inflammatory myopathies characterized by muscular weakness and inflammation. Some patients also present with characteristic skin changes, interstitial lung disease (ILD) or cancer, among other symptoms. Myositis-specific and myositis-associated autoantibodies (aAbs) have been found in patients. In addition, new specific aAbs have been included in the diagnostic panels.

Objective: To assess the clinical value of the new PM/DM-specific aAbs in defining clinical phenotypes in our patients diagnosed with PM/DM.

Materials and methods: All patients with a suspicion of PM/DM admitted to Hospital Puerta del Mar (Cádiz, Spain) for the last 18 months were tested for anti-TIF1-γ, anti-NXP2, anti-MDA5 and SAE-1 aAbs (New DM/PM-aAbs) by using immunoblot (Euroimmun, Germany). Clinical features were recorded.

Results: New PM/DM-aAbs were detected in 2 patients. Patient 1 had anti-TIF1-γ aAbs and showed a clinical cute cutaneous phenotype. Neither IILD nor cancers were found. Patient 2 had anti-MDA5 aAbs and showed a skin rash with progressive IILD. Patient 3 had Anti-NXP2 aAbs and presented with severe skin changes without IILD or cancer. Patients did not show evident muscular weakness, although muscle enzymes were elevated in patients 2 and 3.

Conclusions: In our patients, anti-MDA5 aAbs was a good marker for IILD and NXP2 aAbs indicated severe skin disease. Neither TIF1-γ nor NXP2 were markers for associated cancer in PM/DM. These results support the role of the new PM/DM-aAbs in defining the clinical phenotype and the prognosis of DM/PM patients.
Poster Presentations

P.C1.06.07
Frequency of anti-nuclear antibody and anti-dsDNA antibodies in subjects of oral addictive habits
M. Kashti,1 N. Afzal1,2 S. Minhas1,2, M. A. Anwar1, F. S. Khan1, S. Jahan1
1University of Health Sciences, Lahore, Pakistan, 2Akhatar Saeed Medical and Dental College, Lahore, Pakistan.

Background: People with addictive habits are prone to both infectious and non-infectious diseases. Conflicting results have been reported about propensity of these individuals for development of autoimmune diseases. Therefore, a study was planned to determine the frequency of anti-nuclear antibody (ANA) and anti-dsDNA (anti-dsDNA) antibody in the serum of habitual smokers, paan (areca nut) chewers and other oral addictive habits as compared to subjects without such addictive habits.

Methods: Blood samples from 80 subjects (45 with addictive habits and 45 without any addiction) were taken by random sampling after getting written informed consent. Enzyme linked immunosorbent assay (ELISA) was used to test the sera for ANA and anti-dsDNA.

Results: One subject in the addictive group had ANA and anti-dsDNA antibodies, whereas in the control group, two subjects had anti dsDNA while none of them had ANA. No significant association of these antibodies was observed between the two groups.

Conclusions: Addictive habits do not predispose the subjects to develop autoimmune diseases.

P.C1.06.08
Functional role of CD83 expressed by FoXP3+ regulatory T cells in the context of inflammatory bowel disease.
C. Koenig, A. Steinkeasser, M. Lechmann
Department of Immune Modulation at the Department of Dermatology, University Hospital Erlangen, Erlangen, Germany.

Inflammatory bowel disease (IBD) is still a significant health problem characterized by chronic and recurrent inflammation of the gastrointestinal tract. Unfortunately, available treatment options for IBD are not satisfactory and therefore, a great need for new and more efficient therapeutic strategies exists. Interestingly, using murine IBD models our group showed that activated Tregs rapidly upregulate CD83 expression and that the application of soluble CD83 ameliorates the clinical symptoms in the DSS-induced colitis model. To investigate the underlying mechanisms, we generated CD83 conditional KO animals (CD83cKO), whereby CD83 expression has been specifically deleted on Tregs only. Using these animals we investigated in detail whether deletion of CD83 modulates the suppressive function and/or development of Tregs in steady state and in the context of IBD. The clinical impact of CD83 expression on Tregs was monitored using histology, weight loss and endoscopy. Phenotypically, these Tregs were characterized by FACS-, RT-PCR- and CBA. Body weight and health status revealed an aggravated colitis in CD83cKO mice which correlated with a highly increased mortality rate in these mice. In addition, elevated expression of pro-inflammatory mediators and reduced numbers of Foxp3+CD103+ and FoXP3+KLRF1+ Tregs were observed in CD83cKO mice. In addition, we are currently investigating the therapeutic potential of SCIDb2 as new strategy for the treatment of IBD by using the murine transfer colitis model.

Our results provide first detailed insights into the mechanistic role of CD83 expressed on Tregs and represent the basis for the development of new specific therapies for patients suffering from autoimmune disorders.

P.C1.06.10
β-Catenin signaling in CD11c+ myeloid cells regulates immune homeostasis in the intestine
C. Kurz1, A. Brand1, F. Bauer1, A. Jiang1, J. Melmann1, J. Ober-Biabaim1, B. C. Clausen1
1Institute of Molecular Medicine, Mainz, Germany, 2Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY 14263, United States, 3Research Oncology, Genentech, South San Francisco, CA 94080, United States.

Introduction: Chronic inflammation in the intestine arises from a loss of tolerance towards harmless environmental antigens like nutrients or commensal bacteria. Myeloid antigen-presenting cells (APCs), including macrophages (MO) and dendritic cells (DC), play essential roles balancing immunity and tolerance. β-Catenin is the central component of the canonical Wnt signaling pathway and has previously been demonstrated to promote a tolerogenic DC phenotype in vitro. Hence, CD11c-specific β-catenin deficiency aggravates disease in a mouse model of DSS-induced colitis, suggesting that β-catenin is a key regulator of myeloid cell function in vivo. Whether β-catenin in APC is able to attenuate intestinal inflammation remains elusive.

Methods: To further investigate the role of CD11c-specific β-catenin signaling in the regulation of intestinal immune homeostasis, we generated mice with either a deletion (CD11c-Bcatfl/fl) or expressing a stabilized form of β-catenin (CD11c-BcatEX3). Subsequently, these mice were analyzed in the steady-state as well as in DSS-induced colitis.

Results: In steady-state mesenteric lymph nodes (mLN), CD11c-BcatEX3 mice exhibited higher numbers of DC and regulatory T cells (Tregs) as compared to controls, whereas DC and Treg numbers in mLN of CD11c-Bcatfl/fl mice remained unchanged. Moreover, CD11c-BcatEX3 mice were less susceptible to DSS-induced colitis accompanied by increased numbers of FoxP3+ Tregs.

Conclusion: Our data indicate that activation of β-catenin signaling in CD11c+ myeloid cells supports their tolerogenic function via the induction of Tregs. In ongoing experiments we are dissecting the molecular mechanism(s) by which β-catenin enables distinct MØ and DC subsets to regulate intestinal immune homeostasis in the steady-state and during inflammation.

P.C1.06.11
A deep analysis of the intestinal immune cell compartment in dextran sodium sulfate (DSS) induced colitis
M. Letizia1,2, Y. Rodriguez Sille1, F. Schmidt1, R. Glouben1, C. Weidinger1, B. Siegmund1, C. Weidinger1
1Charité, Berlin, Germany, Humboldt-Universität zu Berlin, Berlin, Germany, 2ZIB Graduate School Berlin, Berlin, Germany.

Colitis disease is characterized by epithelial barrier breaches and a subsequent translocation of bacteria from the intestinal lumen into the adjacent mesenteric fat, inducing the hyperplasia of adipose tissue as well as the recruitment of various immune cells. However, the functional role of mesenteric fat in intestinal immunity and the immunologic imprinting occurring upon epithelial barrier defects is currently unknown. One of the most used mouse model of experimental colitis employs DSS treatment. Although the immune cell composition of colon lamina propria has been investigated, little is known about DSS induced changes within the mesenteric fat. Therefore, we analyzed the immune cell composition of colon lamina propria in comparison with the mesenteric fat compartment. C57BL/6 mice were fed 2.5% DSS in their drinking water for 5 days to induce acute colitis or 1.5% DSS for 5 days followed by 7 days water in 4 cycles to induce chronic colitis. Immune cells were isolated from mesenteric fat, gonadal fat, mesenteric lymph nodes and lamina propria and analyzed by flow cytometry or mass cytometry. Our data give for the first time a comprehensive characterization of immune cells in colon lamina propria in comparison with the mesenteric fat compartment. C57BL/6 mice were less susceptible to DSS-induced colitis accompanied by increased numbers of FoxP3+ Tregs.

Conclusion: Our data indicate that activation of β-catenin signaling in CD11c+ myeloid cells supports their tolerogenic function via the induction of Tregs. In ongoing experiments we are dissecting the molecular mechanism(s) by which β-catenin enables distinct MØ and DC subsets to regulate intestinal immune homeostasis in the steady-state and during inflammation.

P.C1.06.12
Induction of antibodies to citrullinated protein antigens (ACPAs) by stressed neutrophils
T. Li
Karolinska Institutet, Stockholm, Sweden.

There are very interesting findings published by Mariana I. Kaplan showing that stressed neutrophils can induce mitochondrial ROS, activation of PAD4 and the formation of neutrophil extracellular traps (NETs). They have also shown that NETs containing citrullinated peptides are internalized by fibroblast-like synoviocytes (FLS) through a RAGE-TLR9 pathway, promoting FLS inflammatory phenotype and their up-regulation of major histocompatibility complex (MHC) class II. Once internalized, arthritogenic NET peptides are loaded into FLS MHC class II and presented to antigen-specific T cells. HLA-DRB1*04:01 transgenic mice immunized with mouse FLS loaded with NETs develop antibodies specific to citrullinated forms of relevant autoantigens implicated in rheumatoid arthritis (RA) pathogenesis as well as cartilage damage. These results implicate FLS as notable mediators in RA pathogenesis, through the internalization and presentation of NET citrullinated peptidopeptide the adaptive immune system, leading to pathogenic autoimmunity and cartilage damage. Therefore, it will be interesting to study whether proteins from stressed neutrophils can be used to train the humanized mouse strains to induce ACPA production. To this end, we will use mouse leukotriene A (LXA), a toxin from Aggregatibacter actinomycetemcomitans (Aa), which has been shown to induce hypercitrullination in host neutrophils. We have cultured FLS in vitro successfully and verified that these FLS have the ability to present antigens to CD4+ T cells. Next we plan to immunize DRB1*0401, Nc17+/- mice with cit-peptides loaded FLS and detect whether can induce the production of ACPAs and RA.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 319
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.C1.06.13

Compartimentalized tissue adaptation of human colonic CD4+ T cells

L. Lutter1, D. P. Hoytema von Koninjenburg1, J. T. ter Lindt1, A. Petrelli2, N. R. Lansu1, J. ten Have1, M. van der Wolff1, V. Meij1, H. H. Fidder1, M. Mokry2, B. Oldenburg1, F. van Wijk1

1University Medical Centre Utrecht, Utrecht, Netherlands, 2Rockefeller University, New York, United States, 3IRC5 San Raffaele Scientific Institute, Milan, Italy.

The mucosal barrier of the gut is home to numerous intrapithelial (IE) and lamina propria (LP) T cells adapted to the local environment in order to maintain homeostasis. Recent work on tissue T cells has focused on CD8+ "tissue resident memory cells" (Trm); however, the human colon also harbors different, less characterized, CD4+ T cell populations. Tissue resident T cells have been suggested to play important roles in relapsing-remitting inflammatory diseases including inflammatory bowel disease; hence, we FACs-sorted human CD4+ IE and LP T cell populations for RNA sequencing. We show that LP CD4+CD25+ cells resemble the murine cytotoxic CD8+ IE T cells (e.g. expression of GZMA, GZMB, PRF1+ and IFNG), and LP CD4+CD25- cells are similar to classical regulatory T cells. CD4+CD25+ are similar to CD4+CD25- Trm cell populations with similar adaptive immune responses, and both populations are quite different from their LP counterparts.

P.C1.06.14

Immunological findings in patients with recurrent aphthous stomatitis

J. Petanova1, R. Cermakova1, M. Libanska1, J. Bartova1, Z. Jarasova Zakostelska1, Z. Stehlikova1, H. Hlavackova-Hogenova1, I. Izakovcovova-Holila2, S. Slezakova3, P. Bonilova1, Lutter1

1Central Clinical Hospital in Prague, Institute of Immunology and Microbiology, Prague 2, Czech Republic, 2Charles University, Institute of Immunology and Microbiology, Prague, Czech Republic, 3General University Hospital in Prague, Institute of Clinical and Experimental Dental Medicine, Prague 2, Czech Republic, 4Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic, 5Clinic of Stomatodontology, Institute Shared with St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University, Brno, Czech Republic, 6Department of Pathophysiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, 7Department of Clinical Immunology and Allergology, Institution Shared with St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

The etiology of recurrent aphthous stomatitis is not yet clear, immunopathologic states like autoimmunity, allergy or deficiency are mentioned either. We analyzed the immunological status of 73 patients with recurrent aphthous stomatitis (28 men, 45 women, aged 18-72 years). The following parameters were studied: humoral immunity (serum concentration of immunoglobulin IgG, IgA, IgM, IgE, subclasses, complement components C3 and C4, acute phase protein CRP, selected autoantibodies), cellular immunity (specific number of lymphocytes, T lymphocytes subpopulations, B lymphocytes, natural killer cells). The immunological parameters in cellular immunity were in normal ranges. In humoral immunity we found in 13 patients slightly decreased or increased serum concentrations of IgG (4), IgA (6), IgM (3), elevated IgE levels occurred in 14 patients. Anti-nuclear antibodies were slightly positive or positive in 15 patients (21%). Eighteen (38%) of 48 patients had slightly positive antibodies to desmogleins. The clinical periodontal finding and in some cases mucosal biopsy with histopathological findings excluded autoimmune blistering diseases in all tested patients. We found high inter-individual variability in anamnestic and cellular immunity parameters in patients with recurrent aphthous stomatitis. The positivity of antibodies to desmogleins is not specific, positive results can be found in different inflammatory mucosal or cutaneous states. More specific tests have to be performed together with specialized periodontal clinical findings. The work was supported by Ministry of Health of the Czech Republic, grant nr.15-2936A.

P.C1.06.15

The impact of food antigens on the intestinal homeostasis and inflammatory bowel disease

Y. Rodriguez Sillke1,2

1Charité - Universitätsmedizin Berlin, Medical Department (Gastroenterology, Infectious Diseases, Rheumatology) Campus Benjamin Franklin, Berlin, Germany, 2Institute of Nutrition, University Hospital of Marburg, Germany, 3Institute of Medical Microbiology and Hygiene, University of Marburg, Germany.

One of the hallmarks of inflammatory bowel disease (IBD) is a dysregulation of the intestinal immune system. Although nutritional therapy with the elemental diet proves to be of great interest, little is known about its mechanism. Murine data indicate that food antigens induce an activation and subsequent apoptosis of the CD4+ T-cells in the Peyer’s Patch (PP) thus maintaining the healthy balance of the mucosal immune system.

PP T-cells were characterized for patients of Crohn’s disease (CD) and Ulcerative colitis (UC) as well as healthy controls. Gluten served as a model food antigen. Thus gluten activated CD4+ T-cells in the peripheral blood of these patients were analyzed by a magnetic enrichment of CD14+ cells and a subsequent cytometric antigen-reactive T-cell analysis (ARTE technology).

CD4+ T-cells isolated from PP of CD patients revealed a significantly reduced apoptotic rate compared to UC patients and healthy controls. This was accompanied by an increased expression of the survival marker Bcl-2. Further characterization identified an up-regulation of FoxP3 as marker for regulatory T-cells, as well as the activation marker, Helios in CD patients compared to UC patients and healthy controls. Moreover, there was a higher frequency of gluten antigen-specific T-cells (CD4+CD69+CD25+) in parallel to an enhanced survival of CD4+T cells in the peripheral blood of these patients. This is in line with the observed results in the PP and reflects the peculiarities of disease immunopathogenesis.

P.C1.06.16

Anti-GP2 antibodies in blood and feces of children with inflammatory bowel diseases

A. Toptygina1,2, E. Semnikova1, S. Petrichuk1

1G.N.Gabrichevsky Research Institute for Epidemiology and Microbiology, Moscow, Russian Federation, 2Lomonosov Moscow State University, Moscow, Russian Federation.

Inflammatory bowel diseases (IBD), such as Crohn’s disease and Ulcerative colitis (UC), are associated with considerable morbidity and reduced quality of life. Anti-glycoprotein2 (GP2) antibodies were found in the blood of CD-patients. The aim was to examine the anti-GP2 antibody levels in blood and feces of CD and UC patients. Serum and coproextract probes from 110 children (64 boys and 46 girls) aged 12.3(2.6-17.9) years old were examined: 36 CD-patients, 30 UC-patients, and 44 control patients with intestinal dysbiosis (DB). IgG and IgA anti-GP2 antibodies were tested by ELISA. Cut-off calculated for children’s IgG anti-GP2 antibodies for IBD versus non-IBD was 13.8U/ml (sensitivity 82.6%, specificity 88.1%). Among 36 CD-patients 10(27.7%) had IgG and 15 (41.7%) had IgA anti-GP2 antibodies. In 30 UC-patients 5(16.6%) had IgG and 10(33.3%) had IgA anti-GP2 antibodies. None of 44 DB-patients had IgA and only 1(3.3%) had IgG (15.5U/ml). The level of anti-GP2 antibodies in the serum of CD-patients was significantly higher in comparison to UC-patients (p<0.05) and control group (p<0.01). The feces levels of anti-GP2 IgG were elevated in CD(23.5U/ml) and UC(20.45U/ml) patients versus 1.99U/ml in DB (p<0.01). Differences in anti-GP2 IgG and IgA profiles between IBD and DB patients reflect the peculiarities of disease immunopathogenesis.

P.C1.06.17

In situ expression of IgA and IgG in intestinal mucosa of Algerian patients with inflammatory bowel disease

R. Toumi1, J. Soufi2, S. Att Younes3, C. Touil-Boukoffa4

1University of Sciences and Technology Houari Boumediene, Algiers, Algeria, 2Anatomic Pathology Service, Mustapha Pacha Hospital, Algiers, Algeria, 3Algeria, Algeria, 4Clinic of Stomatology, Institution Shared with St. Anne’s Faculty Hospital, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

The intestinal mucosa is home to the largest population of Antibody-secreting plasma cells. The antibodies released by these cells constitute a first line of protection but could also be involved in autoimmune processes. Although the role of immunoglobulins in triggering inflammatory bowel disease (IBD) has not yet been established, there is much data to support this hypothesis. IBD including Crohn diseases (CD) and Ulcerative colitis (UC) is chronic multi-factorial disorder affecting the gastrointestinal. In this study, we investigated by immunohistochemical study the expression of IgA and IgG in the intestines by using immunohistochemical methods. We found significantly high number of IgA+ and IgG+ cells for both categories of patients compared with healthy mucosa (p<0.05). The analysis of IgA and IgG expression at different regions of intestine of the patients did not show a significant difference. However our data, showed the presence of high number of IgA+ cells in the colonic mucosa of patients with CD in comparison to data in the group of patients with recurrent aphthous stomatitis. The positivity of antigen of patients was not specific, positive results can be found in different inflammatory mucosal or cutaneous states. More specific tests have to be performed together with specialized periodontal clinical findings. This study provides additional evidence for the involvement of plasma cells secreting IgG and IgA in the pathophysiology of IBD.
P.C1.06.18

HMGB1 is released from intestinal epithelia damaged by cholela toxin adjuvant contributes to activation of mucosal DCs and induction of intestinal CTLs and IgA

A. Wokobayashi, M. Shimizu, E. Shinya, H. Tokahashi
Nippon Medical School, Tokyo, Japan.

Oral administration of OVA plus cholera toxin (CT), but not the CTA or CTB subunits, induces OVA-specific CD8+ cytototic T lymphocytes (CTLs) in intraepithelial lymphocytes (IELs). The intestinal OVA-specific CTLs were not induced in CD11c+ dendritic cell (DC)-depleted CD11c-DTR mice. CD8+CD103+CD11c+DCs and DCIR2+CD103+CD11c+DCs were distributed in the intestinal lamina propria and mesenteric lymph nodes, both DC subsets expressed DEC-205, and the expression of costimulatory molecules such as CD80 and CD86 was enhanced in both DC subsets after oral administration of intact CT but not the CTA or CTB subunit. Intestinal DCs activated by the oral administration of OVA plus CT cross-presented OVA antigens, and DCs that captured OVA antigen through DEC-205, but not DCIR2, could cross-present antigen. We found that oral administration of intact CT, but not the CTA or CTB subunits, induced cell death, cytotoxic expression of high mobility group box 1 protein (HMGB1) in epithelial cell adhesion molecule (EpCAM)-CD45 intestinal epithelial cells (IECs) and HMGB1 levels in fecal extracts. HMGB1 dose-dependently enhanced the expression of CD80 and CD86 on DCs in vitro, and intravenous or oral administration of glycyrrhizin, an HMGB1 inhibitor, significantly suppressed activation of mucosal DCs and induction of intestinal OVA-specific CTLs and IgA by oral CT administration. These results showed that oral administration of intact CT triggers epithelial cell death in the gut and the release of HMGB1 from damaged IECs and that the released HMGB1 may mediate activation of mucosal DCs and induction of CTLs and IgA in the intestine.

P.C1.06.19

Alterations in immune cell subsets in patients with chronic silicosis caused by artificial quartz agglomerates


1Hospital Universitario Puerta del Mar, Cadiz, Spain, 2Preventive Medicine Service, Cadiz, Spain, 3Hospital Universitario Puerta Real, Cadiz, Spain, 4Public Health Service, Cadiz, Spain,
5Preventive Medicine Service, Cádiz, Spain, 6Preventive Medicine Service, Cadiz, Spain.

Background: Silicosis produced by Artificial Quartz Agglomerates (AQAs) evolves more aggressively than the classical form of miners. This entity is emerging worldwide and a significant group of cases has been detected in the province of Cádiz (Spain) in recent years. The role of the cellular immune response in the pathogenesis of silicosis by AQA has not been previously studied. Objectives: to analyse cell populations present in peripheral blood from patients with silicosis by AQA and compare them with the ones obtained from healthy volunteers. Methods: 48 patients diagnosed with silicosis by AQA and 18 healthy controls were studied. The blood cells populations were quantified by flow cytometry. Results: No differences were found in the total number of leukocytes or granulocytes. However, a significant increase in mononocytes cell number and a clear lymphocytopenia were observed in the blood from patients compared to healthy controls. Almost all the lymphocyte subsets studied - B lymphocytes, T lymphocytes and NK cells (“Natural Killers”) - were decreased compared to healthy controls. Particularly, a significant decrease in the total cell number was observed in the following subsets: memory B lymphocytes, Th helper lymphocytes, naive and memory T-lymphocytes, T-regulatory lymphocytes and CD56+ NK cell subpopulations. However, there was a significant increase in the TH1 and TH17 subsets as well as in plasma cells in patients. Conclusions: These alterations in blood cell populations could reflect different states of inflammatory and fibrotic activity in these patients.

P.C1.07.01

Maintenance and local regulation of tissue specific immunity - Part 7

P.C1.07.02

G protein-coupled receptor 15 is associated with smoking and relapsing remitting multiple sclerosis

C. Ammitzboll, M. R. von Essen, L. Börnsen, E. Petersen, O. McWilliam, H. B. Søndergaard, F. Selebjerg

Danish Multiple Sclerosis Center, Copenhagen, Denmark.

Background: Smoking is an established risk factor associated with autoimmune diseases including multiple sclerosis (MS). We have studied the effect of smoking on circulating immune cells and found GPR15 mRNA expression upregulated in healthy smokers and patients with relapsing remitting MS (RRMS). The protein expression of GPR15 was related to smoking but not RRMS. GPR15 encodes a G protein-coupled receptor on lymphocytes, which together with the recently identified GPR15L, is involved in immune homeostasis in the skin and colon. Objectives: to study GPR15 and GPR15 expressing cells in the cerebrospinal fluid (CSF) of smokers and patients with RRMS. Results: By flow cytometry we found higher frequencies of GPR15+ T cells in CSF compared with blood. In smokers, blood- and CSF frequencies of GPR15+ T cells were increased and were in the CSF associated with chemokine receptors CCR6 and CXCR3 compared with GPR15- T cells. EUSA analyses showed lower concentrations of GPR15 in CSF than in blood and concentrations were unaffected by smoking or RRMS. In patients with RRMS, GPR15+ cells were positively correlated with CD4+GPR15+CCR6+CXCR3+ T cells, melanin basic protein (MBP) and functional disability measured by the expanded disability status scale (EDSS). Conclusion: In the present study we suggest a novel cell type in MS pathogenesis, linked to smoking. Smoking increases circulating GPR15+ T cells with CNS migration potential (CCR6 and CXCR3 expression). In patients with RRMS, these cells (from the CD4+ T cell population) are associated with GPR15L in the CSF, damage of the myelin sheaths and functional disability.

P.C1.07.03

The HLA-DR3 peptide repertoire in epithelial cells versus the conventional antigen processing

M. R. von Essen, L. Börnsen, E. Petersen, O. McWilliam, H. B. Søndergaard, F. Selebjerg

Danish Multiple Sclerosis Center, Copenhagen, Denmark.

HLA-DR3 (HLA-DR17, DRB1*0301/DRA1*0101) is an HLA-DR allele associated to a high number of autoimmune diseases, including type 1 diabetes or Graves’ disease. Previous work on HLA-DRB1*0301-positive Graves’ thyroid tissues revealed the presence of peptides from tissue-specific antigens in the HLA-DR-associated repertoire. The peptide repertoires of human D3+ spleen samples were then processed and compared with thyroid tissue. The results showed a clear tissue-specific bias in the spleen repertoire, where a large frequency of peptides derived from diseased tissues. In addition, the overall affinity of the peptides from the spleen was higher than that of thyroid peptides. In order to identify possible mechanisms of differential HLA-II processing in MHC-II epithelial cells versus canonical APCs, peptide repertoires were studied from a human HLA-DR3, -Ia- and -DM transfected rat insulinoma epithelial cell line compared with a homogenous D3+ lymphoblastoid B cell line. The results showed different differences between both repertoires in parameters such as affinity or hydrophobicity. Tissue specificity was evident in the epithelial repertoire. We identified several peptides derived from proteins restricted to neuroendocrine tissues, including TIMU autoantigen ICAM2 or autoimmune uveitis-related calpain-5. In contrast, the peptides found in EBV were mostly derived from ubiquitous proteins. Interestingly, a high frequency of cytotoxic-degraded peptides was found in the DR3 repertoires, both from epithelial cells, B-LLCs and spleen cells, to a maximum of 40% in the spleen samples. This contrasts with the B-LLC repertoires associated to most HLA-DR alleles, which usually contain around 20% of such peptides.

P.C1.07.05

Rapid isolation of functional ex vivo human skin resident memory T cells

W. Du, C. Cendoré, A. Schueler, E. Zhang, J. Badoi, H. Chang, A. Radbruch, J. Dong

1German Rheumatism Research Center Berlin, Berlin, Germany, 2Sankt Gertrauden Krankenhaus, Berlin, Germany, 3Plastische und Ästhetische Chirurgie, Berlin, Germany.

Introduction In mice, skin-resident memory T (TRM) cells have been shown to provide rapid local protection. To further dissect human skin TRM cells, various isolation approaches have been applied, e.g. EDTA isolation, collagenase F digestion, and skin explants, however, these protocols either suffer from low yield or require long ex vivo culture periods. We established a modified collagenase IV digestion protocol for rapid isolating high yield viable TRM+ cells while preserving intact epitopes of interest.

Materials and Methods Skin samples were obtained from healthy donors under plastic surgeries. Subcutaneous fat were removed and the remaining tissue was minced and incubated in digestion medium at 37°C for 6 hours. Subsequently, the digested skin fragments were dissociated and the cell suspension was filtered and stained with various surface markers. Viable cells were counted and the expression of CD45 and resident memory T cell markers were analyzed. In parallel, this protocol was compared with other protocols such as whole skin dissociation and collagenase F digestion.

Results and Conclusions In terms of yield and cell viability, the modified collagenase IV digestion protocol resulted in at least 1.5 times more T cells per cm² with relatively higher viability than other protocols. Moreover, this modified protocol could well preserve critical surface marker expressions (e.g. CD4, CD8 and CD69), as opposed to other protocols. Therefore, the modified collagenase IV digestion protocol is suitable for further functional assays which acquire relative high amount of viable skin T cells, providing an opportunity for better understanding of human skin TRM cells.
**POSTER PRESENTATIONS**

P.C1.07.06
FOXO1 activity controls CD8 T cell effector function and prevents live immune pathology during viral hepatisis and non-alcoholic steatohepatitis

M. Dudzik
Institute of Molecular Immunology and Experimental Oncology, Munich, Germany.

**Introduction:** Upon antigen-recognition, cytotoxic T-lymphocytes (CTLs) eliminate infected cells. Cellular co-regulation of metabolism and immunity is known to influence effector function, but the mechanisms controlling organ-specific immunity in tissues rich in nutrients such as liver remained unclear. Here we identify FOXO1-activity in CTLs as critical regulator of their metabolic activation during liver disease states.

**Material and Methods:** Extracellular flux analysis, cytokine expression, cytotoxicity assays were performed to study CTL co-regulation of metabolism and immunity and its dependence on FOXO1. Marine models of viral hepatitis and non-alcoholic steatohepatitis (NASH) were used to explore FOXO1-dependent T cell immunopathology.

**Results:** We previously discovered that FOXO1 was required for memory CD8 T cell differentiation through cross-priming liver-sinusoidal-endothelial cells by downregulating NASH. Here we report that, FOXO1-activity also controlled metabolism in effector CTLs. Upon FOXO1-inhibition, CTLs showed augmented metabolic activity, increased gene-expression of nutrient transporters, augmented nutrient uptake, upregulation of IFN-γ, GzmB and FasL and enhanced effector functions. Adaptive-transfer of FOXO1-inhibited virus-specific CTLs into mice with viral infection of the liver caused liver immunopathology but failed to control viral infection demonstrating dysbalance between antigen-recognition and execution of effector function. In NASH, hepatic CTLs showed decreased FOXO1-levels and increased cytokotic potential. Since RNA sequencing did not reveal presence of particular hepatic CTL-clones in NASH, we assume that increased CTL metabolic activation in absence of FOXO1-control caused hepatic immunopathology.

**Conclusion:** Our results provide evidence for a critical role of FOXO1 in controlling metabolic CTL-activation required to maintain tissue homeostasis and prevent immune pathology but also to allow for functional tissue immune-surveillance.

P.C1.07.07
The regulation of ZCH12A and ZCH12B expression during sterile neuroinflammation

A. Kaszo1, D. D. Biswal1, A. S. Gupta1, M. Wawro1, T. Kordula1
1Faculty of Biochemistry, Biophysics and Biotechnology, Cracow, Poland, 2Virginia Commonwealth University, Richmond, United States.

During sterile neuroinflammation, microglia and astrocytes become activated and release a plethora of pro-inflammatory cytokines such as IL-1β and IL-6. We asked whether activation of astrocytes also triggers a feedback mechanism, which depends on the synthesis of RNases to degrade pro-inflammatory transcripts. We focussed on the regulation of expression of two proteins from ZCH12 family, namely ZCH12A/MCP1P1 and ZCH12B/MCP1P2. The amount of mRNA for Zch12a and Zch12b was analyzed in the spinal cords of LPS-treated mice as well as during experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis. In both experimental models, the level of Zch12a was increased whereas the level of Zch12b was decreased. Similarly, treatment of mouse microglia and astrocytes with IL-1β or LPS or human astrocytes with IL-1β, elevated expression of ZCH12A. However, pro-inflammatory stimuli did not change the level of ZCH12B. Moreover, these ZCH12 family members were actively modulating the course of experimental autoimmune encephalomyelitis. We found that the expression of ZCH12A and ZCH12B up-regulated expression of IL-1β and IL-6, and IL-1β-activated astrocytes. Thus, mechanisms, which induce expression of pro-inflammatory cytokines, also activate expression of ZCH12A, which elicits a feedback mechanism by regulating pro-inflammatory transcripts turn-over. Regulation of ZCH12A activity during neuroinflammation remains elusive and needs further investigation.

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P.C1.07.08
Txf-induced MDCSs alter the early systemic pro-inflammatory response and inhibit the antigen-specific T-cell proliferation

Y. Husecken1, M. Kustermann1, M. Huber-Lang1, K. Debatin1, G. Strauß1
1Department of Pediatric and Adolescent Medicine, 89075 Ulm, Germany, 2Department of Trauma Surgery, Hand, Plastic and Reconstructive Surgery, 89075 Ulm, Germany.

**Introduction:** Txf activates a strong inflammatory immune response, which is counterbalanced by immunosuppression characterized by an impaired adaptive immune response. Inflammation triggers the induction of myeloid-derived suppressor cells (MDCSs) heterogenous population of immature myeloid cells, which suppress various T-cell functions. Thus, induction of MDCSs after infection degrades blunt chest trauma (Txf) and their subsequent influence on innate and adaptive immunity was investigated.

**Methods:** C57BL/6 mice underwent a blast wave to induce Txf. At 6 h to 5 d after Txf, increase in MDCSs in lung and spleen and their suppressive capacity in MLR and in vivo after SEB activation was determined. Alterations in the secretion of cytokines and chemokines caused by Txf-induced MDCSs were analyzed in bronchoalveolar lavage (BAL) fluid and blood sample serum.

**Results:** In Txf animals, MDCS numbers were preferentially enhanced in the lung until 48h after trauma, while total numbers and lymphocyte composition in spleen and lung were not affected. Although, no strong increase in MDCSs was detected, splenic MDCSs isolated after Txf suppressed the proliferative capacity of antigen-stimulated T-cells in vitro. Moreover, trauma-induced MDCSs inhibited T-cell proliferation of SEB activated T-cells in vivo and they supported the production of Th1-associated cytokines. Txf-induced MDCSs decreased the systemic levels of IL-6, G-CSF and MCP-1 but didn’t substantially influence the expression of pro-inflammatory factors in BAL fluid.

**Conclusions:** Thus, our results indicate that Txf promotes the induction of T-cell suppressive MDCSs, which might contribute to trauma-induced immunosuppression.

P.C1.07.09
Development of tissue-resident mucosa-associated invariant T (MAIT) cells in human renal fibrosis and chronic kidney disease (CKD)

B. M. P. Low1, R. Wilkinson1, X. Wang1, K. Kildey1, K. Giulian1, K. Beagley1, H. Healy1, A. J. Kassianos1, 2
1Royal Brisbane and Women’s Hospital, Brisbane, Australia, 2Queensland University of Technology, Brisbane, Australia.

**Introduction:** MAIT cells are a specialised lymphocyte population associated with chronic inflammatory disorders in peripheral tissues. To date, MAIT cell research has focused primarily on mucosal tissue, with limited studies on non-mucosal organs such as kidneys. In this study, we evaluated MAIT cells in native human kidneys with tubulointerstitial fibrosis, the pathological hallmark of CKD. MAIT cells were identified, enumerated and phenotyped from human kidney tissue by multi-colour flow cytometry. Localisation of MAIT cells were performed by immunofluorescence microscopy. MAIT cells and human proximal tubular epithelial cells (PTEC) were cultured under hypoxic (1% O2) conditions to examine mechanistic tubulointerstitial interactions. We detected significantly elevated numbers of MAIT cells (Tcr-β2.1+ CD161+) in diseased biopsies with interstitial fibrosis compared with diseased biopsies without fibrosis and healthy kidney tissue. The increased numbers of MAIT cells correlated significantly with loss of kidney function (eGFR). MAIT cells in fibrotic biopsies expressed development markers (IL-7Rα, IL-18Ra), activation receptor (NKGD2), extravasation marker (CD44) and tissue-resident markers (CD69, CD103, and CD49a). Immunofluorescent staining of fibrotic kidney tissue localised the accumulation of MAIT cells within the tubulointerstitial compartment, adjacent to PTEC. Notably, PTEC under in vitro pro-fibrotic/hypoxic conditions up-regulates tissue-resident markers CD69 on MAIT cells. We provide the first characterisation of MAIT cells in human kidney tissue. Collectively, our data suggest that human MAIT cells are retained as tissue-resident lymphocytes and are positioned to contribute the fibrosis process via complex interactions with PTEC. Further dissection of kidney MAIT cells offers a novel pathway of disrupting the mechanisms of CKD.

P.C1.07.10
Eomescontrols the development of Th17-derived (non-classical) Th1 cells during chronic inflammation

A. Mazzoni1, L. Maggi1, F. Siracusa1, M. Ramazzotti1, M. Rossi1, V. Santarlasci2, G. Monta1, M. Capone1, B. Rossetti1, R. De Palma1, A. Kruglov1, H. Chang1, R. Ciampi1, E. Maggi2, S. Romagnani1, F. Liotta1, L. Casini1, F. Anunziato1
1University of Florence, Florence, Italy, 2German Rheumatism Research Center, Berlin, Germany, 3University of Company, Naples, Italy.

**Introduction:** Th17 cells are a highly plastic cell subset that can be easily directed towards the Th1 phenotype in vivo. Knockout of Eomes in Th17 cells, which are a mouse model of inflammatory disease, led to the development of classical Th1 cells. However, the mechanism by which Eomes controls Th1 development was unclear.

**Methods:** We generated Eomes△-/- mice, which allow to reduce Eomes expression in vivo. We used these mice to examine the effects of Eomes deficiency on Th1 development in vivo.

**Results:** We showed that Eomes deficiency led to the development of classical Th1 cells. Furthermore, we found that Eomes deficiency led to the development of classical Th1 cells in vivo. These results suggest that Eomes expression is required for the survival of Th1 cells in vivo. The mechanism by which Eomes controls Th1 development is currently under investigation.

**Conclusion:** Our results provide evidence that Eomes expression is required for the survival of Th1 cells in vivo. The mechanism by which Eomes controls Th1 development is currently under investigation.
Human lymphoid tissues harbor, in addition to circulating CD56− and CD56+ natural killer (NK) cells, a third NK cell population: CD69+CXC6+ lymphoid tissue (t)NK cells. To obtain more insight in the characteristics of tNK cells, RNA sequencing was performed on the three NK cell populations from bone marrow and blood. 700-900 genes were differentially expressed between individual populations in blood or marrow. Among the downregulated tNK cell genes, we identified SDF1, SELP4 and SEL2. By flow cytometry we confirmed that the adhesion molecule (e.g. CD49e) and transcription factor profile (e.g. GZMB, GZMH, GNLY) of tNK cells differed significantly from their circulating counterparts. tNK cells were characterized by enriched expression of inhibitory receptors TIGIT and CD96, and low expression of DNAM1 and cytolytic molecules (e.g. GZMB, GZMH, GNLY). Their proliferative capacity was reduced compared to the circulating NK cells. Gene set enrichment analysis revealed the transcription factor EGR2 and phosphatase DUSP6 as potential regulators of the tNK cell transcriptome. Remarkably, comparison of the tNK cell and published human spleen-resident memory CD8− Trm cell transcriptomes identified tNK cells as a highly enriched subset of bone marrow CD8− Trm cells. Together, we provide molecular data that clearly distinguish tNK cells from both the circulating CD56− and CD56+ NK cells and substantiate the view that tNK cells are tissue-resident and functionally restrained in killing. Our findings underscore the existence of a core gene signature shared between CD8− Trm and resident NK cells in lymphoid tissues.

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P.C1.07.16
Apoptosis of food-activated T cells in Peyer’s patches is a hallmark of healthy intestines

L. Steinhoff1, S. Hartmann1, K. Rajapakse1, B. Siegmund2, Y. Rodriguez Silke3, R. Glabach1, A. Visserunza1
1Institute for Microbiology and Hospital Hygiene, Marburg, Germany, 2Institute for Immunology, Mainz, Germany, 3Charité-Medical Department for Gastroenterology, Berlin, Germany.

It is well known that development of the intestinal immune system is shaped by the microbiota. However little information exists about the impact of dietary antigens on development and homeostasis of the intestine. We studied the impact of dietary proteins on the fate of intestinal immune cells. Data from mice and humans show that continuous exposure to dietary protein antigens leads to highly activated Helios+ Foxp3+ CD4+ T cells in Peyer’s patches (PP), independently of the intestinal microbiota. Food protein activated, Helios+ CD4+ T cells in PP undergo PD-1 mediated apoptosis and removal of dead cells by macrophages induces local IL-10 production. Further studies in patients with inflammatory bowel disease revealed significantly reduced frequencies of apoptotic CD4+ T cells as compared to healthy controls. These findings demonstrate that continuous activation and subsequent apoptosis of diet-reactive CD4+ T cells is a hallmark of the healthy intestine.

P.C1.07.17
Age dependent changes in T cell subsets in multiple sclerosis patients

A. Tejeda Velarde1, E. Rodríguez-Martín1, L. Costa-Frassard2, Y. Aladro3, S. Sainz de la Maza4, J. Fernández4, S. Medina4, N. Villarrubia5, E. Monreale6, J. Álvarez-Cerrillo6, E. Roldán7, L. Medina8
1Ramón y Cajal University Hospital, Madrid, Spain, 2Getfes University Hospital, Madrid, Spain.

Introduction: T cells have an important role in multiple sclerosis (MS) pathophysiology. CD4 trigger the inflammatory cascade and recruit immune cells into the central nervous system, while CD8 contribute to induce axonal damage. However, age dependent changes in these T cell subsets remain unknown. We aimed to study age dependent changes in CD4 and CD8 subsets in cerebrospinal fluid (CSF) and blood of MS patients.

Patients and methods: We analyzed CSF and blood of 102 MS patients (91 relapsing-remitting and 11 primary progressive) during gestation-dependent shifts. We observed a well-defined B cell population in 1 trimester of pregnancy, while the percentage of T cells in placenta rises after >17wk gestation. Various subpopulations of NK cells might reveal key mechanisms of how placental immunity is maintained.

Results: In blood, we observed an age dependent decrease in naive CD8 (r=-0.41; p<0.0001), accompanied by an increase in effector CD8 (r=0.35; p=0.0006), especially in TD CD8 (r=0.33; p=0.0011). We also found an increase in PD1+ CD4 (r=0.48; p=0.0035). These associations were not found in CSF. However, in the CSF we observed an increase in both CD4 (r=0.34; p=0.0017) and CD8 (r=0.25; p=0.0273) Treg. We did not find any association with Treng, but we observed an increase in CD4 PD-1+ (r=0.56; p=0.0146) in the CSF.

Conclusions: Our data show that the distribution of T cell subsets in CSF and blood change along the time in MS, and this could have some implications in the prognosis of these patients.

P.C1.08.01
Uterine immune dynamics assessed from pre-pregnancy endometrium to delivery

M. Benner1, D. Feyaerts1, C. Cartagena Garcia2, G. Ferwerda3, W. Shadmehr4, O. W. van der Heijden5, J. Joosten6, R. G. Van der Molen7
1Roadst Institute for Molecular Life Sciences, Nijmegen, Netherlands, 2Mildred Clinics, Arnhem, Netherlands.

Introduction: The importance of local immunity for correct placental development and healthy pregnancy is evident. Various studies focusing on a particular cell type and time points during pregnancy suggest gestational immune shifts. However, methodological differences between studies make it impossible to fully assess when and to what extent changes in placental immune signature are induced. Here, we examined >30 different lymphocyte (sub)populations and their dynamics during gestation. Methods: Deep immune flowcytometry phenotyping was performed on lymphocytes isolated from the uterine mucosa throughout gestation. Pre-pregnancy endometrial lymphocytes, isolated from menstrual blood, lymphocytes from placenta at different time points (S-13 6wk, 12-14wk and >17wk) and peripheral blood mononuclear cells were processed immediately. Results: NK cells were enriched in endometrium up until the 2nd trimester of pregnancy, while the percentage of T cells in placenta rises after >17wk gestation. Various subpopulations of NK and T cells revealed gestation-dependent shifts. We observed a well-defined B cell population in 1st and 2nd trimester placentae, with highest levels in the 2nd trimester. Especially CD24+CD38+ B cells were increased in 2nd trimester decidua. Currently, we are assessing the nature and putative specificity of these cells. Conclusion: Uterine mucosal immunity changes extensively after the induction of pregnancy. Gestation-dependent local B cell function deserves attention. Contrary to a common belief that decidual B cells are almost absent or only present in pathological conditions, our results highlight possible functional implications in early to mid gestation. Investigation of decidual tolerance-mediating B cells might reveal key mechanisms of how placental immunity is maintained.
POSTER PRESENTATIONS

P.C1.08.02
A novel human CD3'CD56' regulatory cells: role in the pathogenesis of type 1 diabetes

1Istituto per l’Endocrinologia e l’Oncologia Sperimentale- Consiglio Nazionale delle Ricerche, Naples, Italy, 2University of Naples “Federico II”, Naples, Italy, 3Istituto di Ricovero e Cura a Carattere Scientifico Multimedica, Milan, Italy, 4University of Potenza, Potenza, Italy.

Regulatory T cells play a cardinal role in the control of immune response and homeostasis. Here, we reported that a circulating T cell population, co-expressing CD3 and CD56 molecules (denoted as CD3'CD56'), identifies a novel human regulatory T cell subset, not expressing the fork-head box-P3 (Foxp3) transcription factor and distinct from classical Treg cells. Flow-sorted CD3'CD56' cells showed that CD3'CD56' cells were able to suppress cytotoxicity and IFNγ production when co-cultured in vitro with CD8+ effector cells. Regulatory function of human CD3'CD56' cells required cell-to-cell contact and associated to a reduction of intracellular reactive oxygen species (ROS) in CD8+ target cells. Furthermore, through a microarray analysis, we characterized the transcriptome profile of CD3'CD56' cells, clustering independently from other circulating T cell subset. Finally, these data were validated in human autoimmune condition; indeed, peripheral frequency and suppressive function of CD3'CD56' cells were significantly reduced in a large cohort of children with type 1 diabetes, at diagnosis. Taken together, our findings unveil a previously unrecognized regulatory T cell population specifically controlling CD8+ effector functions, which may play a critical role in the control of immunological tolerance and autoimmune diseases in humans.

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P.C1.08.04
Tissue specialization of human Tfr cells
R. Correa1, V. R. Fonseca2, A. Agua-Doce1, L. Gaczi1;

1Instituto de Medicina Molecular João Lobo Antunes, Lisboa, Portugal, 2Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisboa, Portugal, 3Instituto Gulbenkian de Ciência, Oeiras, Portugal.

The recent discovery of a follicular regulatory T (Treg) cell subset, called T follicular regulatory (Tfr) cell, has revealed a new and specialized mechanism for germinal centre (GC) regulation and prevention of autoimmunity. Tfr cells gain access to the GC and suppress follicular helper T (Tfh) cells and B-cell responses. In humans, blood Tfr cells are not fully characterized and expression of immunoregulatory molecules is still controversial. We studied Tfr cells in human lymph nodes and tonsils. We found that the presence of GC reactions impacts the phenotype of Tfr cells. While in human lymph nodes without GC reactions (identified by Bcl-6 immunohistochemistry), Tfr cells resemble their blood counterparts, GC presence is associated with the emergence of activated Tfr cells, as the ones usually found in human tonsils.

Taken together, these results suggest that human Tfr cells follow a tissue compartment specialization accordingly to the presence of ongoing humoral responses. A detailed phenotypical and functional analysis of Tfr cells in different human tissues is critical to understand the biology of these cells.

P.C1.08.05
Potential role of CD4+T cell-derived extracellular microRNAs in the loss of immune tolerance during multiple sclerosis
S. Garavelli, F. Battarì, F. Carboni, C. Procaccini, V. De Rosa, D. Centonze, G. Maresare, P. De Candia;

1IRCCS Multimedica, Milan, Italy, 2IRCCS Istituto Neuromedico Mediterraneo (INM) Neuromed, Pozzilli, Italy, 3Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNCR), Naples, Italy.

Introduction: CD4+ regulatory T (Treg) cells control inflammation by inhibiting the growth and cytokine production of CD4+ T conventional (Tconv) cells. We showed that, upon stimulation, T cell subsets release extracellular vesicles (EVs), containing distinct patterns of microRNAs with the capability of modulating specific mRNA targets upon cellular uptake. Objective of the present work was to evaluate whether the T cell-derived extracellular microRNA signatures may get dysregulated in autoimmunity with a potentially different impact on the transcriptome of EV receiving cells. Materials and Methods: To this aim, human CD4+ T cell subsets were purified from peripheral blood of naive to treatment relapsing-remitting multiple sclerosis (RRMS) patients and healthy controls. Tconv- and Treg cells were in vitro stimulated and released EVs were characterized by nanoparticle tracking analysis and RT-qPCR to analyze size distribution and microRNA content. Results: Compared to healthy, both Tconv and Treg cells from patients showed a differentially expressed set of microRNAs with crucial regulatory function in the immune system. By a mRNA profiling approach, we have then demonstrated that the treatment of naive T cells with Treg (but not Tconv) EVs caused the specific repression of genes involved in the proteasome-dependent proteolytic process, known to be crucial for T cell activation, and that in RRMS, Treg-derived EVs may have lost this capability. Conclusions: Our results unveil a novel molecular mechanism for Treg-mediated maintenance of self-tolerance and its potential alteration in multiple sclerosis. Grants: National Multiple Sclerosis Society NMS5 (PP-1606-24687) and Fondazione Italiana Sclerosi Multipla FISM (2016/R/10) to PdC.

P.C1.08.06
The role of regulatory T cells in the development of obesity in MIF-KO mice
D. Gajić, J. Koprivica, M. Vujičić, I. Stojanović, T. Zaksida;

Institute for Biological Research “Siniša Stanković”, Belgrade, Serbia.

Obesity is a disorder characterized by a pro-inflammatory environment in visceral adipose tissue (VAT) due to increased infiltration of pro-inflammatory macrophages and a drop in regulatory T (Treg) cells. Macrophage migration Inhibitory Factor (MIF) is a pro-inflammatory cytokine with versatile functions in innate and adaptive immunity. Although it has a key role in the immune system, its innate absence in MIF-KO mice is larger in mass and the infiltration of amelanotic cells per gram of VAT is not different than in WT controls. Also, MIF-KO VAT has the same distribution of immune cells (CD3+, CD4+, CD8+, CD19+ cells, M1 and M2 macrophages), but a higher expression and secretion of TNF-α and IL-1β. Surprisingly, Treg cells are more abundant in VAT of MIF-KO mice. Proliferation of Treg cells in VAT measured by BrdU incorporation (BrdU-I) was not different than in WT VAT, while the number of CD4+CD25+FoxP3+ Treg cells was significantly larger in MIF-KO VAT compared to WT VAT. Based on these results, we can assume that Treg cells in VAT of MIF-KO mice are, albeit extensively present, less functional. This situation may be responsible for obesity development in the absence of MIF. Supported by Ministry of Education, Science and Technological Development, Republic of Serbia (172013).

P.C1.08.07
Exploratory study on the association of Vγ9Vδ2 Tcells with Chlamydial cervico-vaginal infection in women with Recurrent Spontaneous Abortions

1Dept. of Immunology and Histocompatibility “Helena Venizelou” Hospital, Athens, Greece, 22nd Dept. of Internal Medicine, Aristotel University of Thessaloniki, 3Hospital GKT, Thessaloniki, Greece, 4Dept. of Microbiology “Helena Venizelou” Hospital, Athens, Greece.

Background: We have previously reported a significant association of Vγ9Vδ2 T cells with cervic/vaginal Chlamydial trachomatis(Ct)infection in women with RSA. This association raises questions on the chlamydial antigens that activate γδ T cells to develop an anti-trophoblast response as well as the factors that enhance it. In the present study, we try to answer the γδt6-mediated anti-trophoblast activity is related to the length of the infection by analyzing the γδt6+SCCs in aborters with or without received anti-clamydia treatment.

Patients-Methods: The percentage of γδt6+SCCs in PB was analyzed by flow cytometry in 76 positive for Ct infections (Ct+) RSA women (A), within 10 days after a new miscarriage. 35 of them had received anti-Ct treatment upon diagnosis (T) and 41 were not treated (NT). Fertile women without Ct infection (Ct-) served as controls (C). Results: A highly statistically significant difference of mean percentages of γδt6+SCCs between was shown between Tand C groups (58.95 vs 61.92, p<0.0001). The analysis performed in T and NT aborters revealed that the mean percentage was 58.95% and 83.29% respectively (p=0.0002) and that 53% of T women (n=20) of γδt6+SCCs (only 20% of T women, p=0.0017).

Conclusions: The increased levels of PB γδt6+SCCs in untreated cases support the hypothesis that the presence of Ct in the cervic/vaginal tract favors the recognition by γδt6+SCCs of trophoblastic antigens sharing common epitopes with antigens (HSP7) expressed on the embryonic tissues which results in the activation of anti-embryonic responses and possibly abortions.

P.C1.08.08
Sertoli cells have non-canonical functional inflammomassacre network able to perturb tests niche function and to inflict cell death
K. Todorova, E. Avramova, L. Seer, A. Apostolova, S. Haymann;

Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Sertoli cells are pivotal in protecting auto-immunogenic germ cells by an immune privileged environment, but eventually they could also serve as germine damage sentinels. We have recently shown a functional Nrpi3 inflammomassacre in mouse Sertoli cells, both primary and cell line, where challenging of NOD1/NOD2 and TL4, in ATP presence activated caspase-1, incurring cell death and mature IL-1β, IL-6, IL-23 secretion. We explored if the caspase-1 mediated cell death was pyroptosis-like and it was only Nrp1 regulated.
Surprisingly, we found an even larger non-canonical innate immunity inflammasome network elicited by TLR4/NOD2/ATP challenge (NGS RNA-Seq: intact Sertoli vs. Ma, Illumina; challenged vs. intact Sertoli cells, Oxford Nanopore), that was preserved in metabolic stress (caspase-1, RT-qPCR) and consisted of several inflammasomes and adaptors: Caspase-1 inflammasomes Alm2 (9000 fold), Nlr10 (7000 fold), Nlrp3 (200 fold) and Nlrc5 (100 fold) upregulation overwhelmed anti-apoptotic Naip1 (100 fold). The importance of metabolic preference for glycolysis (Agiilent Seahorse) was supported by the role of MAPK3 for NF-κb upregulation upon challenge (MAPK3 siRNA in stable 15P-1 cell line with pNFMy2-SEAP reporter). Sertoli cells undergo partial pyroptosis-like cell death (PI imaging, flow cytometry), with membrane leakage (LDH assay), blebbing (live imaging, fluorescent reporter), but without pyroptosis-like membrane shedding.

Inflammasome network serves as sentinel for germline genome stability by sensing DAMP/PAMP/metabolic stress, acting through caspase-1 axis, a noton coinciding with clinically observed semen IL-18/IL-18 finding in male infertility patients, suggesting new therapy avenues.

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P.C1.08.09
The alarminIL-33 draws a ST2+ T-reg mediated anti-inflammatory immune response during immune-mediated hepatitis
F. Heinrich, A. Ochel, G. Teggs, K. Neumann;
University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Introduction: During Concanavalin A (ConA)-induced immune-mediated hepatitis, the alarmin IL-33 is released by necrotic hepatocytes and induces immune responses by signaling through the IL-33 receptor ST2. We have shown previously that IL-33 pre-treatment prevents development of immune-mediated hepatitis suggesting an immunosuppressive function for IL-33 in liver disease. Since regulatory T cells (Tregs) respond to IL-33, we aimed to investigate the IL-33 driven Treg response in the inflamed liver.

Methods: C57BL/6 mice were treated either with IL-33 or PBS alone or received ConA after 3-day IL-33/PBS treatment. Mice were analyzed 24 hours after hepatitis induction. The phenotype of Foxp3+ Tregs was determined by flow cytometry.

Results: We showed that the frequency of hepatic Foxp3+ Tregs was significantly increased in IL-33-treated mice compared to PBS-treated mice. Within the Treg population, the frequency of Tregs expressing ST2 was highly elevated. Compared to ST2+ Treg, hepatic ST2+ Tregs displayed an activated phenotype with up-regulated expression of CD25, KLRL1, and ICOS as well as enhanced expression of the functional markers CTLA-4, TIGIT, and GITR. During immune-mediated hepatitis, the frequency of hepatic ST2+ Tregs was elevated compared to healthy mice and was further strongly increased by IL-33 pre-treatment. Interestingly, hepatic ST2+ Tregs also have an activated phenotype in liver inflammation that was not altered by IL-33 pre-treatment.

The immunosuppressive function of IL-33 in immune-mediated hepatitis might be driven by expansion and recruitment of a highly activated Treg population expressing ST2.

P.C1.08.10
Dendritic cells as predictors for embryo implantation
K. Kyvelidou, A. Geisler, B. Toth, C. Heufers, S. Kofer-Tollerig; Medical University of Innsbruck, Innsbruck, Austria.

The immunological processes surrounding implantation and pregnancy are still under investigation. Promising players are dendritic cells (DC), major controllers of the immune system and key immune cells in the human endometrium. We hypothesize that the mechanisms for the establishment of maternal tolerance are generated even before implantation, are highly dependent on DC for their initial activation, and that embryodo-produced bioactive factors trigger DC for tolerance. Discarded small medium (SM) from human single-embryo cultures was collected in order to perform a large scale protein array and also to be used for the treatment of the DC. Overall was to compare the differences between medium from embryos that resulted in pregnancy (pSM) and the ones that did not (npSM). Preliminary results so far show a different protein expression pattern in the SM which can be associated with a positive or a negative pregnancy outcome. Furthermore, several DC genes were found to be regulated by the SM. VCAM-1, CXCL2, CCL19, CXCL8, RELB, and NFKB1 were found upregulated by the pSM, while CCL2 was found upregulated by the npSM. These results indicate that indeed preimplantation embryos are able to produce specific factors that can affect DC. The elevated number of DC during pregnancy and their ability to interact with T regulatory and natural killer cells render DC a very promising target in the field of maternal tolerance and their study can offer new insights in the immunological events surrounding implantation and early pregnancy and inspire novel immunomodulatory strategies and treatment options for infertility.

P.C1.08.11
Myeloid-specific molecular mediators of subchloral bone damage in antigen-induced arthritis
N. Lucač,1 M. Fadljević,1 I. Radanović,1 E. Lazic Mosler1,4, A. Sucur1,4, D. Flegar1,4, I. Tkalčev1,4, V. Katavci4, D. Grcević1,4, N. Kocic1,4;1Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia, 2Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia, 3General Hospital Dr. Ivo Pidolišić, Sisak, Croatia, 4Catholic University of Croatia, Zagreb, Croatia, 5Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia.

Introduction: Rheumatoid arthritis (RA) is marked by subchondral bone destruction, joint deformations and disability. Analable therapeutics improve the prognosis, but with limited effect on bone destruction. Using antigen-induced arthritis (AIA), animal model of RA, we found that mice deficient for Fas gene (Fas-/-) develop non-destructive arthritis, marked by lower frequency of myeloid cells in joints. We aim to identify mediators of bone resorption in arthritis, by analyzing differentially expressed genes in sorted myeloid population from wild-type (WT) and Fas-/- mice with AIA.

Materials and methods: AIA was induced by intra-articular injection of methylated bovine serum albumin to immunized mice. Bone resorption was assessed by µCT. Synovial cells were released by collagenase, labeled with anti-mouse CD45-FITC, CD11b-PE, Gr1-PECy7, B220/CD3/NK1.1/CD31/TER119-APC, and CD51-APCeF780. CD11b+Gr1+ population was sorted using BD FACSAria. RNA extracted by TriZol was hybridized to Affymetrix ST 2.0 arrays. Differences in gene expression found on arrays were confirmed by qRT-PCR. Results: Synovial CD11b+Gr1+ cells were transcriptionally similar in Fas-/- and WT mice with AIA. Samples split into two hierarchical clusters consisting predominantly of Fas-/- or WT samples. WT-dominant cluster revealed up-regulated genes related to cell cycle progression and mitosis, suggesting higher proliferation. Mid1 and Erdr1 genes were down-regulated. In WT samples. WT-dominant cluster revealed up-regulated genes related to cell cycle progression and mitosis, suggesting higher proliferation. Mid1 and Erdr1 genes were down-regulated. In WT samples.

Conclusion: Resorptive AIA is marked by higher myeloid proliferation potential. Mid1 gene is a potential novel mediator for targeting inflammation-mediated joint destruction in arthritis. Grants: This work was supported by the Croatian Science Foundation project number 7406.

P.C1.08.12
Functional analysis of inflammation associated parameters in a patient with NEMO deficiency
N. Sürüşçü,1,2 B. Kayaoglu,1,2 E. Alpdündar Bulut,1,3 J. C. Ayanoğlu,1,3 E. Dündagoğlu,1,3 M. Acar,1,2 B. Sözeri,1,3 A. Kıykım,1,3 E. Karakoş- Aydınler,1,3 S. Barsız,1,3 A. Özen,1,3 M. Gürsel1,3;1Middle East Technical University, Department of Biological Sciences, Ankara, Turkey, 2Health Sciences University Umraniye Teaching and Research Hospital, Pediatric Rheumatology Clinic, Istanbul, Turkey, 3Marmara University Hospital, Pediatric Allergy and Immunology Department, Istanbul, Turkey, 4Marmara University Hospital, Pediatric Allergy and Immunology Department, Istanbul, Turkey.

NEMO (NF-κb essential modulator, IKK-γ), is a regulatory component of the IKK (inhibitor of NF-κb) kinase complex and has a central role in the activation and subsequent translocation of the nuclear factor (NF)-κb transcription factor. NEMO deficiency, is a rare type of primary immune deficiency which has also been associated with autoinflammatory manifestations. This study aimed to investigate underlying mechanisms of inflammation in a patient with NEMO deficiency that presented with recurrent fever, panniculitis and intestinal neutrophilic infiltrates and nodular skin lesions. PBMC and neutrophils were isolated from peripheral blood and used for functional assays and gene expression analysis using the NanoString inflammatory panel. PBMC of the patient and healthy controls were stimulated with various TLR ligands or anti-CD3/anti-CD28. Results showed decreased cytokine responses in the patient (IL-1β, IL-6, IL-17, IFNy) compared to healthy individuals. However, type I IFN responses to cytosolic nucleic acids was uncompromised. Low density granulocytes (LDG) in the PBMC fraction were abnormally increased in the patient and gene expression analysis subsequently confirmed a striking increase in MMP9 expression. Nanostring pathway analysis showed a decreased score for IKK and NF-κb signaling pathway but an upregulation of type I interferon related genes MX1 and IFI44 and an elevated JAK/STAT pathway score. Consistently, cytometric bead array (CBA) analysis of plasma showed a sixty-fold increase in circulating IP-10 levels in the patient compared to healthy controls. Collectively, these results suggest that inflammation-associated symptoms in the patient might stem from elevated type I interferons and LDGs, leading to granulocyte dysregulation.

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326 Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Hypoxia and rheumatoid phenotype decrease the T helper cell suppressive capacity of synovial fibroblasts

1University of Heidelberg, Heidelberg, Germany, 2German Cancer Research Center (DKFZ), Heidelberg, Germany.

Introduction: The pathogenesis of rheumatoid arthritis (RA) is linked to functional changes in synovial fibroblasts (SF) and local infiltration of T lymphocytes. Increased synovial inflammation is also associated with a hypoxic microenvironment. Oxygen levels in the joints of RA patients are significantly decreased (mean oxygen tension: 3.2%) compared to those of osteoarthritic (OA) patients. So far, little is known about the effects of hypoxia on the interaction between fibroblasts and T cells and its implications on the pathobiology of RA. Methods: SF of OA or RA patients were co-cultured with Th cells under normoxic or hypoxic (3% O2) conditions. Th cell proliferation was determined by flow cytometry, cytokine secretion by ELISA, indoleamine 2,3-dioxygenase 1 (IDO1) expression was analysed by Western Blot and real-time PCR, tryptophan/kynurenine levels were quantified by HPLC. Results: Under normoxic conditions, SF strongly inhibited the proliferation of co-cultured Th cells by an IDO1-mediated depletion of tryptophan. RA SF showed a significantly lowered IDO1 expression, tryptophan metabolism and a significantly weaker capacity to suppress the proliferation of Th cells compared to OA SF. Under hypoxic conditions, the SF of OA and RA were both suppressed in Th cell suppressive capacity of SF and RA were significantly reduced. Conclusions: The IDO1-mediated suppression of Th cell growth may play an important role in preventing inappropriate Th cell responses under normal conditions. The reduced tryptophan metabolism under hypoxia together with the inferior efficiency of RA SF to restrict T cell proliferation likely supports the development of synovitis in RA.

Increase of aerobic glycolysis mediated by activated T helper cells drives synovial fibroblasts towards an inflammatory phenotype

P. Kvacskay, M. M. Souto-Carneiro, R. Carvalho, K. D. Kika, H. Lorenz, L. Tylovcanská
1University of Heidelberg, Heidelberg, Germany, 2University of Coimbra, Coimbra, Portugal, 3German Cancer Research Center (DKFZ), Heidelberg, Germany.

Introduction: There is growing evidence for a dysregulated glucose metabolism of synovial fibroblasts (SF) in rheumatoid arthritis (RA) being a prerequisite for their aggressive phenotype. As yet, little is known about the influence of immune cells on the metabolism of SF although local infiltration of leucocytes constitutes a hallmark in the pathogenesis of RA. Here, we investigated the effect of Th cells on the glucose metabolism and cytokine production of non-inflammatory (osteoarthritids (OA)) and inflammatory (RA) SF. Methods: RASF and OA SF were cultured in the presence of a stable glucose isotope ([U-13C] Glucose) and stimulated with culture supernatants (SN) of activated Th cells. Glucose and lactate levels were determined by proton magnetic resonance spectroscopy (1H-NMR). Cytokine secretion was quantified by ELISA. Results: Resting RA SF showed a significantly higher production of lactate. IL-6 and MMP-3 compared to OA SF. Stimulation by activated Th cells strikingly changed the metabolic profile of both SF by inducing a shift towards aerobic glycolysis with strongly increased lactate production. In parallel, a significant increase in IL-6 and MMP-3 secretion was observed. Interestingly, blocking of either IL-6 or of glycolysis significantly reduced both the production of lactate and the secretion of inflammatory cytokines. Conclusions: A Th cell-mediated metabolic switch towards aerobic glycolysis in SF in RA could likely be an important step in the pathogenesis of RA. Targeting this metabolic switch by ablation or of glycolytic inhibitors may provide a new strategy in RA therapy.

Mammary gland involution stimulates severe local inflammation that promotes breast cancer tumourigenesis

A. Unsworth, R. Anderson, N. Haynes, R. Britt
1Peter MacCallum Cancer Centre, Department of Onecology, Melbourne, Australia, 2Olivia Newton-John Cancer Wellness & Research Centre, Heidelberg, Australia, 3Sir Peter MacCallum Cancer Centre, Department of Oncology, Melbourne, Australia.

During reproduction the breast undergoes significant structural changes to prepare for breastfeeding. Once breastfeeding ceases, the mammary gland undergoes rapid tissue remodeling, which is linked to a wound-healing response. While this process is tightly controlled, the wound-healing environment during involution is also known to be ideal for tumor development. Preliminary transcriptional analysis suggests the immune system may play a critical role in increasing breast cancer risk during involution, as inflammatory markers are significantly up-regulated in the involuting mammary gland. Our study aims to examine the specific composition of immune cells in the mouse mammary gland during reproduction, specifically involution (repair) and parous (resting) using a multi-panel flow cytometry approach and cytokine bead analysis. To determine the effects of these reproductive environments on breast tumour development, syngeneic mammary tumor cells were introduced orthotopically into involuting, parous and nulliparous mice. The involuting mammary gland exhibits a significant increase in myeloid cells, coupled with decreases in T and B lymphocytes and expansion of pro-inflammatory cytokines compared to nulliparous glands. This inflammatory environment stimulates mammary tumor growth at a faster rate compared to that of a resting nulliparous gland. The parous mammary gland (assessed 9-weeks post-involution) showed complete resolution of this inflammatory microenvironment, consequently having no effect on tumor growth rate in mice. The involuting mammary gland exhibits a strong inflammatory microenvironment, which we show promotes the development of breast cancer. We are currently investigating immune targeted therapeutics that could be used to reduce this local inflammation and consequently decrease breast cancer risk during involution.

Human myometrial CD4 T cells at the maternal-fetal interface are tissue-resident memory T cells, which show site-specific adaptation within the uterus

J. Wienk, J. van der Burgh, T. E. Vogelvang, A. Fransen, B. V. Van Rijn, F. van Saar-Wijtse
1UMC Utrecht, Utrecht, Netherlands, 2Diakonessenhuis, Utrecht, Netherlands.

Introduction: The uterine myometrium is a unique immune environment, capable of harbouring a ‘foreign’ fetus without eliciting an immune response. We aimed to investigate the presence, adaptation and function of human myometrial CD4 T cells at the maternal-fetal interface in uncomplicated pregnancy. Methods: Myometrial biopsies were obtained at caesarean section from placental and incision site. Lymphocytes were isolated through digestion and digestion with collagenase IV. CD4 T cells were analysed by flow cytometry or FACS sorted for RNA sequencing by CEL-seq protocol. Suppression assays were performed with FACS sorted uterine regulatory T cells (Tregs). Results: 70-80% of myometrial CD4 T cells were CD69+, suggesting a tissue-resident memory (TRM) phenotype. RNA sequencing confirmed a TRM-like profile in CD69+ cells, with high expression of CD49a, CXCR6, DUSP6, PD-1 and low expression of CD62L, KLF2/3 and S1PR1 compared to blood memory CD4 T cells. Interestingly, expression of negative costimulatory molecules such as PD-1, TIGIT, Lag3, TIM-3 and CTLA4 on the CD4 T cells, as well as the percentage of Treg cells, was higher at the placental site compared to the maternal site. Conclusion: Myometrial CD4 T cells at the maternal-fetal interface are TRM cells with a high expression of negative costimulatory molecules and a high abundance of functional Tregs. Preliminary transcriptional analysis suggests the immune system may play a critical role in increasing breast cancer risk during involution, as inflammatory markers are significantly up-regulated in the involuting mammary gland. This inflammatory environment stimulates mammary tumor growth at a faster rate compared to that of a resting nulliparous gland. The parous mammary gland (assessed 9-weeks post-involution) showed complete resolution of this inflammatory microenvironment, consequently having no effect on tumor growth rate in mice. The involuting mammary gland exhibits a strong inflammatory microenvironment, which we show promotes the development of breast cancer. We are currently investigating immune targeted therapeutics that could be used to reduce this local inflammation and consequently decrease breast cancer risk during involution.

Defective Regulatory T cells and B cell Subsets Are Associated with Autoimmunity in Common Variable Immunodeficiency Patients

R. Yazdani, G. Azizi, H. Abolhassani, F. Kiasse, A. Mirshafiey, A. Aghamohammadi
1Research Center for Immunodeficiencies (RCID), Tehran, Iran, Islamic Republic of.

Introduction: Common variable immunodeficiency (CVID) is one of the most prevalent symptomatic primary immunodeficiencies (PIDs), which manifests a wide clinical variability such as autoimmunity, as well as T cell and B cell abnormalities. Methods: A total of 72 patients with CVID were enrolled in this study. Patients were evaluated for clinical manifestations and classified according to the presence or absence of autoimmune disease. We measured regulatory T cells (Tregs) and B-cell subsets using flow cytometry, as well as specific autoantibody response (SAR) to pneumococcal vaccine, autoantibodies and anti-IgA in patients. Results: Twenty-nine patients (40.3%) have shown at least one autoimmune disease, including autoimmune cytopenias, autoimmune gastrointestinal diseases and autoimmunity in 27.8% of patients. 38.5% and 79.3% presented a defect in Tregs and switched memory B-cells, respectively, whereas 69.0% manifested. Autoimmune cytopenias and autoimmune gastrointestinal diseases were the most common. A significant association was detected between autoimmunity and increased synovial inflammation is also associated with a hypoxic microenvironment. Oxygen levels in the joints of RA patients are significantly decreased (mean oxygen tension: 3.2%) compared to those of osteoarthritic (OA) patients. So far, little is known about the effects of hypoxia on the interaction between fibroblasts and T cells and its implications on the pathobiology of RA. Methods: SF of OA or RA patients were co-cultured with Th cells under normoxic or hypoxic (3% O2) conditions. Th cell proliferation was determined by flow cytometry, cytokine secretion by ELISA, indoleamine 2,3-dioxygenase 1 (IDO1) expression was analysed by Western Blot and real-time PCR, tryptophan/kynurenine levels were quantified by HPLC. Results: Under normoxic conditions, SF strongly inhibited the proliferation of co-cultured Th cells by an IDO1-mediated depletion of tryptophan. RA SF showed a significantly lowered IDO1 expression, tryptophan metabolism and a significantly weaker capacity to suppress the proliferation of Th cells compared to OA SF. Under hypoxic conditions, the SF of OA and RA were both suppressed in Th cell suppressive capacity of SF and RA were significantly reduced. Conclusions: The IDO1-mediated suppression of Th cell growth may play an important role in preventing inappropriate Th cell responses under normal conditions. The reduced tryptophan metabolism under hypoxia together with the inferior efficiency of RASF to restrict T cell proliferation likely supports the development of synovitis in RA.
P.C.01.08.18
Regulatory function of B cells on T cell immunity during viral infection is controlled by the surface receptor Toso
J. Yu, N. Füger, K. Lee;
Inflammation Research Group, Institute of Clinical Chemistry, Hannover Medical School, Hannover, Germany.

The immune system is tightly controlled by regulatory processes that allow for the elimination of invading pathogens, while limiting immunopathological damage to the host. In this study, utilizing conditional gene deletion, we demonstrate a critical immunoregulatory role of the cell surface receptor Toso, that, via a B cell-inherent mechanism, provides protective T cell immunity against viral infection. Employing conditional gene deletion, our study reveals that impaired anti-viral T cell responses in Tosο deficient mice, were not due to T cell intrinsic defects, but rather inherent to a previously unrecognized function of Tosο in B cells. We specifically demonstrated that the deletion of Tosο in B cells results in impaired inflammatory T cell responses, such as production of TNFα and IFNγ, in response to influenza A infection. Further studies showed that Tosο deficiency in B cells results in a strong increase of IL-10 competent B cells in vivo, and, as we further demonstrate through adoptive transfer experiments, this specific subtype of B cells mediates immunosuppressive activity on T cell immunity during influenza A infection. Thus, Tosο exhibits its immunoregulatory function by controlling a pool of IL-10 competent regulatory B cells. In addition, we demonstrate that during influenza A-induced pulmonary inflammation the application of Tosο-specific antibodies selectively induces IL-10 competent B cells at the site of inflammation and results in decreased proinflammatory cytokine production by lung T cells. These findings suggest that Tosο may serve as a novel therapeutic target to dampen pathogenic T cell responses via the modulation of IL-10 competent regulatory B cells.

P.C.01.08.19
Roquin deficiency in T cells induces early stages of pancreatic cancer
T. Raj1, J. Zöller1, D. Hu1, G. Bianco2, M. Heikenwälder1, V. Heissmeyer1,2;
1Biomedical Center Munich, Institute for Immunology, LMU Munich, Planegg - Martinsried, Germany; 2Department of Chronic Inflammation and Cancer, German Cancer Research Center, Heidelberg, Germany, *Research Unit Molecular Immune Regulation, Helmholtz Zentrum München, Munich, Germany.

Our immune system not only prevents cancer development by eliminating transformed cells, it can also drive chronic inflammation causing tissue insult and the development of cancer. Acute and chronic pancreatitis are strongly implicated in the development of pancreatic ductal adenocarcinoma (PDAC), the most frequent form of pancreatic cancer and one of the leading causes of cancer deaths worldwide. Investigating mice with combined Roquin-1 and Roquin-2 deficiency in T cells we show that chronic T cell activation can lead to autoimmune pancreatitis, acinar-to-duct metaplasia (ADM) and formation of PanIN (pancreatic intraepithelial neoplasia) lesions in mice. Pancreatic damage increases with age, with half of the mice aged 10 - 20 weeks exhibiting precursor lesions. Pancreata were infiltrated with inflammatory cells and pSTAT3 levels were strongly increased, pointing towards a potential role of IL-6 mRNA deregulation in T cells during the development of Roquin mediated pancreatic pathologies. These mice produced autoantibodies against pancreatic antigens, suggesting an involvement of Tfh cells. Combined genetic activation of the Roquin encoding alleles as well as of its target Oxa40 lead to a partial rescue and an improved phenotype. Currently, we are investigating the contributions of Tfh and Th17 cells to the pancreatic pathology. Furthermore, we are evaluating antibodies against Roquin dependent surface antigens as potential biomarkers for PDAC in mice and humans.

We propose that mice with conditional Roquin deletion in T cells are a useful model to study how chronic inflammation and autoimmune pancreatitis triggers the development of neoplasia and pancreatic cancer.

P.C.01.08.20
Expression pattern of TIPE1 insights into its functions
S. Liu, J. Shao, Y. Li, G. Jin, C. Gao;
Department of Immunology, A’nan, China.

Members of the tumor necrosis factor-alpha-induced protein-8 (TNFAIP8 or TIPE) family play important roles in immune homeostasis and cancer. TIPE1 (TNFAIP8-like 1) is a new member of the TIPE family that may regulate cell death. We found that TIPE1 protein was detected in a wide variety of tissues in C57BL/6 mice and a variety of cells of the epithelial origin, particularly with secretory functions. High levels of TIPE1 mRNA were detectable in most human carcinoma cell lines. TIPE1 is also detectable in endothelial cells, which can induce endothelial dysfunction when exposed to oxidative stress and therefore result in arteriosclerosis in Apoe/-/- mice.

P.C.01.08.21
Characteristics of gamma/delta T cells at the feto-maternal interface of murine pregnancy
J. M. Nörenberg1, M. Meggyesi1,2, P. Jakl1, A. Barakonyi3;
1Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Pécs, Hungary, 2János Szentgáthai Research Centre, Pécs, Hungary, 3Department of Pathology, Medical School, University of Pécs, Pécs, Hungary.

Pregnancy is an immunological enigma where paternal antigens are present at the feto-maternal-interface. What regulates the local immunotolerance which is necessary to prevent rejection of the conceptus is still under strong investigation. Gamma/delta T cells are believed to play a role in the local regulation of this immunotolerance towards the allogeneic fetus. Gamma/delta T cells from uterus and spleen of pregnant and non-pregnant mice were analyzed by flow cytometry. The ratio of γδ T cells in the decidua associated lymphoid tissue increases during the course of murine pregnancy. Those decidual γδ T cells are in large part γδTCR3+/CD44-, whereas γδTCR3+ cells are mainly CD4+.

Furthermore, compared to peripheral γδ T cells, a greatly proportion of decidual γδ T cells express CD107α, TIM-3 and TIM-1, by contrast no difference in the expression of CD160 with γδ T cells from the spleen. Within lymphocytes expressing CD107α, TIM1 or CD160, the rate of γδ T cells is significantly higher in the decidua. Accordingly, γδ T cells seem to influence the contributions of Th1 and Th17 balance, which is crucial to provide protection against pathogens, while mediating tolerance towards the semi-allelograft. This study was supported by a grant from the University of Pécs (AOIK-KA/13-03/30439) and by TÁMOP 4.2.4. A/2-11-1-2012-0001. The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary.

P.C.02.01.01
Immune signaling and therapy in autoimmunity - Part 1

P.C.02.01.02
The anti-inflammatory neuropetide cortistatin plays a critical role in the development and progression of atherosclerosis
R. Benítez1, I. Forte-Lago1, C. Gao1, F. O’Valle1, M. Delgado1,2,3;
1Institute of Parasitology and Biomedicine Lopez Neyra, CSIC, Granada, Spain, 2Department of Pathology, School of Medicine, University of Granada, Granada, Spain.

Atherosclerosis is a chronic organ-specific autoimmune disease that causes important adverse circulatory events and is responsible of high mortality worldwide. Cortistatin is a neuropetide produced by immune and auto immune cells that plays a role in the vascular system and atherosclerotic plaques that inhibits inflammation in different experimental models of autoimmune diseases, including atherosclerosis. Here, we investigated whether a deficiency in cortistatin predisposes to suffer exacerbated atherosclerosis by using established preclinical mouse models. We generated mice that lack of apolipoprotein E (apoE-) and are totally (C57+ KO) or partially (C57+ Het) deficient in cortistatin. Mice were subjected to cardiac partial ligation and fed a hyperlipidemic diet for four weeks to induce acute localized plaques, or alternatively were fed a normal or hypercholesterolemic diet for various weeks to induce chronic atherosclerotic plaques in aorta and aortic sinus. We observed that apoE−/− and apoE+C57−/− mice developed higher (number and size) atherosclerotic plaques in carotid artery, heart, aortic arch and aorta than apoE−/− mice fed a high-lipid diet. Interestingly, total or partial deficiency in cortistatin predisposed significantly to increased mortality during the progression of this disease.
Even with normal diet, apoe−/−CST-kio and apol−CST-het mice showed early severe atherosclerotic plaques in heart and aortic arch. Lack of cortistatin did not change serum cholesterol, but increased the presence of lipid-loaded macrophages and of TH1 and TH17 cells in plaques and draining lymph nodes and of inflammatory M1 macrophages in peritoneum. Our findings demonstrate the endogenous role of cortistatin in the regulation of potential predisposition to inflammatory cardiovascular disorders.

P.C2.01.04
The effect of HLA on autoantibody isotypes & cytokine production in myasthenia gravis with autoantibodies to muscle specific tyrosine kinase (MuSK-MG)
M. CEBE, H. DURMUŞ, S. YENTUR, V. YILMAZ, F. AYSAŁ, Y. PARMAV, P. ÖZFAZER, F. DEYMEER, G. SARUHAN-DİRİŞEKELİ;
1 Istanbul University, Istanbul University Medicine Faculty, Department of Physiology, İstanbul, Turkey, 2 Izmir University, Izmir University Medicine Faculty, Department of Neurology, İstanbul, Turkey, Babürký Sadi Konuk State Hospital, İstanbul, Turkey.
A small subset of myasthenia gravis (MG) develops with autoantibodies against muscle-specific kinase (MuSK). These anti-MuSK autoantibodies are predominantly of IgG4 isotype. MuSK-MG is strongly associated with HLA-DQA1*0501 with HLA-DRB1*14 or HLA-DRB1*16. In this study, the effect of these HLA-associations on the anti-MuSK IgG autoantibody isotype and antibody-related cytokine production was investigated. Among all patients with MG who were followed at the Neurorheumatic Unit of Istanbul University Medical Faculty, 80 patients with anti-MuSK antibodies were selected. Disease-associated HLA types were detected in the collected DNA samples. Anti-MuSK-IgG1, IgG2, IgG3 and IgG4 antibody tiers and levels of IL-6, IL-17A and IL-10, measured by ELISA, compared between the groups with or without HLA-DQA1*0501, HLA-DRB1*14 or HLA-DRB1*16 by non-parametric tests. Anti-MuSK-IgG4 tiers were significantly higher than -IgG1, -IgG2 and -IgG3 isotypes (p<0.001, p<0.001, p<0.001) in the whole group of patients. Anti-MuSK-IgG1 tiers were also relatively higher than -IgG2 and -IgG3 tiers (p<0.001). When the tiers were compared between HLA subgroups, DRB1*14 (+) MuSK-MG patients had higher IgG4 and IgG1 tiers than DRB1*14 (-) patients (p=0.017, p=0.002). Higher IL-10 (>0.048) and lower IL-17A (>0.011) levels were measured in DRB1*14 (+) patients compared to DRB1*14 (-) patients. No other differences were detected.

P.C2.01.05
A diet in fibre can moderate inflammation and kidney pathology in a model of systemic lupus erythematosus
T. A. Gottschalk, E. Tatsiakos, M. L. Hibbs; Monash University, Melbourne, Australia.
Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease mediated by the deposition of immune complexes in tissues including the kidney, with the ensuing inflammatory cascade driving progressive tissue damage and dysfunction. Mice lacking Lyn tyrosine kinase (Lyn-/-) mice develop an autoimmune disease similar to SLE, driven by dysregulation of the immune system, immune complex deposition in tissue and systemic inflammation culminating in progressive glomerulonephritis. The gut microbiome has been shown to have an immunoregulatory effect on the development of autoimmmune and inflammatory diseases, in large part due to the production of short chain fatty acids from the fermentation of dietary fibre. To determine whether dietary fibre could moderate systemic autoimmune and inflammatory pathology, Lyn−/- mice and control C57BL/6 mice were fed a high fibre diet (HFD) or a standard control diet from weaning until 42 weeks old. On the control diet, Lyn−/- mice developed lymphopenia, splenomegaly from enhanced splenic myelopoesis, immune cell hyperactivation, and pathogenic IgG anti-dsDNA autoantibodies resulting in glomerulonephritis. These hallmarks of inflammation and autoimmune disease were significantly moderated in HFD fed Lyn−/- mice, indicating that dietary intervention is effective at dampening chronic inflammation, associated with glomerular pathology and the gut microbiome in regulating systemic immune responses and controlling autoimmunity, inflammation, and preventing the progression of immunopathology and suggests that fibre supplementation may improve outcomes for those living with SLE or other chronic systemic inflammatory diseases. This work was funded by grants from the NHMRC of Australia and Monash CCS.

P.C2.01.06
CD11b regulates inflammation, autoimmunity and associated pathology in a model of systemic lupus erythematosus
T. A. Gottschalk, E. Tatsiakos, M. L. Hibbs; Monash University, Melbourne, Australia.
Systemic Lupus Erythematosus (SLE) is a complex, heterogeneous autoimmune disease characterized by circulating self-reactive antibodies to deposits in tissues including the kidney, driving systemic inflammatory responses and associated pathology. Granulocytes and monocytes/macrophages are key components of the systemic inflammatory response, and CD11b plays a role in regulating and controlling the progression of inflammation and autoimmune disease. This work was funded by grants from the NHMRC of Australia and Monash CCS.

P.C2.01.07
Integration of genome-wide DNA methylation and transcription uncovered aberrant methylation-regulated genes and pathways in the peripheral blood mononuclear cells of systemic sclerosis
1 University of Texas Southwest, Midland, Texas, United States, 2 Xiayang Hospital, Central South University, Changsha, China.
Objectives: the aim is to delineate the interaction network between gene expression and DNA methylation in peripheral blood mononuclear cells (PBMC) from systemic sclerosis (SSc) patients and to identify methylation-regulated genes involved in the pathogenesis of SSc. Methods: Genome-wide mRNA transcription and global DNA methylation analysis were performed on PBMC from 18 SSc patients and 19 matched normal controls (NC) using Illumina BeadChips. Differentially expressed genes (DEGs) and differentially methylated positions (DMPs) were integratively analyzed to identify methylation-regulated gene expression patterns.

Results: Transcriptome analysis distinguished 453 DEGs (269 up- and 184 down-regulated) in SSc from NC. Global DNA methylation analysis identified 925 DMPs located on 618 genes. Integration of DEGs and DMPs revealed 20 potential methylation-regulated DEGs (MeDEGs), including 12 up-regulated genes (ELANE, CTSG, LTBR, C3AR1, CSTA, SPI1, ODF3B, SAMD4A, PLAUR, NFE2, ZYX and CTSZ) and eight down-regulated genes (MMELP, CD83, RPS6, CSF1R, COL4A1, LGALS3, FOSL1, NDUFAF1), which clearly distinguished SSc from NC with 100% accuracy. In silico bioinformatics analysis of MeDEGs identified 513 potential target genes of MeDEGs and their associated pathways. Conclusion: Transcriptome analysis distinguished 453 DEGs, 20 potential methylation-regulated genes and 513 potential target genes of MeDEGs.

P.C2.01.08
C1q restrains autoimmunity and viral infection by regulating CDB T-cell metabolism
G. Ling, G. Crawford, N. Buang, X. Zuo, 1 University of Texas Southwest Medical Center, Dallas, United States, 2 Xiayang Hospital, Central South University, Changsha, China.
Objectives: the aim is to delineate the interaction network between gene expression and DNA methylation in peripheral blood mononuclear cells (PBMC) from systemic sclerosis (SSc) patients and to identify methylation-regulated genes involved in the pathogenesis of SSc. Methods: Genome-wide mRNA transcription and global DNA methylation analysis were performed on PBMC from 18 SSc patients and 19 matched normal controls (NC) using Illumina BeadChips. Differentially expressed genes (DEGs) and differentially methylated positions (DMPs) were integratively analyzed to identify methylation-regulated gene expression patterns.

Results: Transcriptome analysis distinguished 453 DEGs (269 up- and 184 down-regulated) in SSc from NC. Global DNA methylation analysis identified 925 DMPs located on 618 genes. Integration of DEGs and DMPs revealed 20 potential methylation-regulated DEGs (MeDEGs), including 12 up-regulated genes (ELANE, CTSG, LTBR, C3AR1, CSTA, SPI1, ODF3B, SAMD4A, PLAUR, NFE2, ZYX and CTSZ) and eight down-regulated genes (MMELP, CD83, RPS6, CSF1R, COL4A1, LGALS3, FOSL1, NDUFAF1), which clearly distinguished SSc from NC with 100% accuracy. In silico bioinformatics analysis of MeDEGs identified 513 potential target genes of MeDEGs and their associated pathways. Conclusion: Transcriptome analysis distinguished 453 DEGs, 20 potential methylation-regulated genes and 513 potential target genes of MeDEGs.
POSTER PRESENTATIONS

P.C2.01.09

Exploiting annexin-mediated immunosuppression to induce antigen-specific tolerance
C. S. Link, F. Bujanji, H. Wayd, P. H. Krommer; German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany.

Apoptotic cells mediate immunosuppression of dendritic cells (DC) and inhibit immune responses. Thereby, apoptotic cells facilitate the induction of peripheral tolerance and the prevention of autoimmunity diseases. We investigate the influence of apoptotic cells on DC and identified the cell surface exposure of the evolutionary conserved annexin core domain (Anx) as a specific signal, which binds to specific receptors on DC and antagonises Toll-like receptor signalling. To further test this tolerogenic capacity of Anx for potential development of antigen-specific immune responses, we generated beads harbouring Anx as well as the model antigen ovalbumin (OVA). Treating BMDC with either Anx-OVA beads (OAB) or with OVA-beads (OB) as a control and subsequent co-culture with OVA-specific CD4+ T cells, we could show diminished T cell activity in the presence of Anx. Furthermore, Anx leads to reduction of cytokine secretion (IL-2, IFN-γ) and of proliferation that suggests induction of T cell anergy by the OAB treatment. These results indicate that coupling of Anx and a defined antigen or allergen in a therapeutic bead preparation might be used as a new approach to downregulate pathologic immune responses in the context of autoimmunity and allergy.

P.C2.01.10

Selective inhibition of gelatinases in CD4+ T-cells reduces clinical severity in a murine model of multiple sclerosis
L. Onwula-Ekpete, D. Takimoto-Roschy, G. B. Fields; Florida Atlantic University, Jupiter, United States.

Introduction: MMP-2 and MMP-9 are the gelatinase members of the matrix metalloproteinase (MMP) family of proteolytic enzymes that mediate the degradation of extracellular matrix components. MMPs are essential for normal physiological processes, but their dysregulation is associated with various pathologies including multiple sclerosis (MS). Experimental autoimmune encephalomyelitis (EAE) is a well-established murine model of MS. In EAE, CD4+ T-cells activated in the periphery penetrate the blood-brain barrier (BBB) to enter the CNS where they initiate destruction of the myelin sheath, and cause axonal loss. The gelatinases are required for these various processes. Recent studies have implicated the gelatinases in normal T cell activation; however, the mechanism of action is not known. Materials and Methods: For in vitro assays, CD4+ T cells were activated with CD3/CD28 mAbs beads after prior treatment with a gelatinase inhibitor. For in vivo assays, EAE mice were treated daily from Day 7 with vehicle or inhibitor and disease progression monitored. Results: Upon activation, CD4+ T-cells treated with inhibitor demonstrated a reduced ability to enter cell cycle, proliferate, and produce cytokines. In addition, RNA-seq analysis on positively regulating autoimmune T cell and negatively regulating immunosuppressive T regulatory cell function. The net result of these two opposing effects may exacerbate clinical severity. Conclusion: In our studies, we demonstrated that the gelatinases are important for homeostatic maintenance as well as a robust antigenic stimulation. These results emphasize the role of gelatinases as therapeutic targets in CD4+ T-celled mediated immune diseases.

P.C2.01.11

Hyperglycemia-dependent NF-kappab O-GlcNAcylation acts as a molecular switch regulating T cell and Treg cell function in autoimmunity
P. Ramakrishnan, T. de Jesus, L. Liu; Case Western Reserve University, Cleveland, United States.

Type 1 diabetes is an autoimmune disease associated with hyperglycemia. Adverse pathological effects of hyperglycemia include posttranslational modification of proteins by the sugar N-acetyl glucosamine (GlcNAc) in a process called O-GlcNAcylation. We found that hyperglycemia induces O-GlcNAcylation of NF-kappaB protein-c Rel, which is a critical regulator of T cell function and T cell development. O-GlcNAcylation of c-Rel at serine 350 activates c-Rel-dependent transcription of proautoimmune cytokines and inhibits the expression of T regulatory cell specific transcription factor FOXP3. Thus, c-Rel O-GlcNAcylation may serve as a key regulatory switch with dual, but reciprocal, roles in positively regulating autoimmune T cell and negatively regulating immunosuppressive regulatory T cell function. The net result of these two opposing effects may exacerbate autoimmunity in type 1 diabetes. This study reveals c-Rel O-GlcNAcylation as a disease-dependent novel molecular mechanism regulating autoimmunity and a potential therapeutic target to control autoimmunity in type 1 diabetes.

P.C2.01.12

High-throughput screening for Lck-coreceptor coupling inhibitor
K. Ruppa, V. Horkova, O. Stepanek; Institute of Molecular Genetics of the ASCR, Prague, Czech Republic.

TCR-mediated activation of T lymphocytes is the key event for the initiation of adaptive immune response that is directed against pathogens but also against self-antigens in the case of autoimmunity diseases. The initiation of TCR signaling is promoted by coreceptors CD4 and CD8 that mediate the interaction of TCR signaling complex with Lck kinase which triggers downstream signaling. We developed a functional model to study Lck-coreceptor coupling. Our data show that Lck-coreceptor coupling is especially important for T cell activation by low affinity antigens and that T cell activation increases with increased Lck-coreceptor coupling. Moreover, it was shown that, in contrast to foreign antigens, self-antigens are recognized with low affinity by TCR of autoreactive T cells that escaped negative selection in thymus. Thus, we assume that inhibiting of Lck-coreceptor interaction significantly alters T cell reactions in vitro and in vivo. To further test this tolerogenic capacity of Lck for potential development of antigen-specific immune responses, we generated beads harbouring Lck as well as the model antigen ovalbumin (OVA). For in vitro assays, CD3/CD28 mAb beads were activated with CD3/CD28 mAb beads after prior treatment with a gelatinase inhibitor. For in vivo assays, EAE mice were treated daily from Day 7 with vehicle or inhibitor and disease progression monitored. Results: Upon activation, CD4+ T-cells treated with inhibitor demonstrated a reduced ability to enter cell cycle, proliferate, and produce cytokines. In addition, RNA-seq analysis on positively regulating autoimmune T cell and negatively regulating immunosuppressive T regulatory cell function. The net result of these two opposing effects may exacerbate clinical severity. Conclusion: In our studies, we demonstrated that the gelatinases are important for homeostatic maintenance as well as a robust antigenic stimulation. These results emphasize the role of gelatinases as therapeutic targets in CD4+ T-celled mediated immune diseases.

P.C2.01.13

Thymol blunts experimental autoimmune orchitis-induced reproductive failure in BALB/c mice
E. Yarahmadi, A. Shalizar-Jalali, G. Najafi, M. Abtahi-Foroushani; Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, Islamic Republic of.

Introduction: Autoimmune orchitis as an autoimmune aggression to testis can result in male subfertility and/or infertility. This study was aimed to scrutinize the possible reproductive-protective activity of thymol (TML) against experimental autoimmune orchitis (EAO)-induced alterations in testicular histoarchitecture and epididymal sperms characteristics and in vitro fertilizing potential in mice.

Materials and Methods: In this study male adult BALB/c mice were randomly categorized to six equal groups including untreated, TML (100 mg/kg BW/day; orally for 5 weeks), antigen (100 μl; subcutaneously) and Bordetella pertussis (10³ bacteria at the day of antigen injection and 48 hours later; intraperitoneally) control groups, EAO and EAO + TML. The EAO was induced through testicular homogenate plus Freund's complete adjuvant and ovalbumin (OVA) injection. All animals were euthanized after 5 weeks and testes histopathological alterations as well as epididymal sperm characteristics and in vitro fertilizing ability were analyzed.

Results: The EAO caused significant reductions in the seminiferous tubules diameter, germinal epithelium height, quality and maturation along with severe testicular morphological alterations including spermatogenic cells maturation arrest, seminiferous tubules depletion and also multinuclear giant cells formation compared to control, TML, antigen and EAO + TML. The EAO was induced through testicular homogenate plus complete Freund's adjuvant plus ovalbumin (OVA) injection. All animals were euthanized after 5 weeks and testes histopathological alterations as well as epididymal sperm characteristics and in vitro fertilizing ability were analyzed.

Conclusions: These findings revealed that TML has repro-protective activities against EAO-evoked disorders in male mice reproductive system. This study was funded by Urmia University, Urmia, Iran.

P.C2.01.14

Paxquinomid prevents development of diabetes in the non-obese diabetic (NOD) mouse

Introduction: Quinoline-3-carboxamides (Q compounds) are immunomodulatory compounds that have shown efficacy both in autoimmune disease and cancer. We have in here investigated the impact of one such compound, paxquinomid, on the development of diabetes in the NOD mouse model for type 1 diabetes (T1D).Methods and Results: In cohorts of NOD mice treated with paxquinomid between weeks 10 to 20 of age and followed up until 40 weeks of age, we observed dose-dependent reduction in incidence of disease as well as delayed onset of disease. Further, in contrast to untreated controls, the majority of NOD mice treated from 15 weeks of age did not develop diabetes at 30 weeks of age. Importantly, these mice displayed significantly less insulin, which correlated with selectively reduced number of splenic macrophages and splenic Ly6C γ inflammatory monocytes at end point as compared to untreated controls. Conclusion: Collectively, these results demonstrate that paxquinomid treatment can significantly inhibit progression of insults to T1D in the NOD mice. We propose that the effect of paxquinomid on disease progression may be related to the reduced number of these myeloid cell populations. Our finding also indicates that this compound could be a candidate for clinical development towards diabetes therapy in humans.
**POSTER PRESENTATIONS**

**P.C2.01.15**

**Autoimmune ShcC/Rai functions the support of autoreactive T cells during EAE by modulating adenosine-dependent CTLA4 expression through the inhibition of CD39 and CD73 activity**

C. Ullevi, D. De Tommaso, F. Fenniri, B. Ortenzi, G. Pellico, M. D’Ellios, C. Ballerini, C. T. Baldari

1University of Siena, Siena, Italy. European Institute of Oncology, Milan, Italy. University “Amedeo Avogadro” Novara, Novara, Italy. University of Florence, Florence, Italy.

Autoimmune T cell recruitment and activation in the central nervous system (CNS) are two recognized pathogenic processes in Multiple Sclerosis (MS). Recent data indicate, however, that the interplay between cells resident in the CNS, including astrocytes, and infiltrated T cells is instrumental for disease onset and progression. In this context, while the impact of astrocytes on the T cell autoimmune response has been partially addressed, how autoreactive T cells modulate astrocytes during experimental autoimmune encephalomyelitis (EAE) has not been explored. We have recently demonstrated that ShcC/Rai is a novel astrocytic adaptor whose loss in mice accounts for a milder EAE notwithstanding a higher frequency of CNS infiltrated autoreactive T cells. Here we have explored and characterized the molecular mechanism that underlies the reciprocal modulation of astrocytes and autoreactive T cells, focusing on the role of ShcC/Rai. Found that astrocytes support autoreactive T cell effector function by upregulating the two purinergic pathways CD39 and CD73 through both contact-dependent and -independent mechanisms. We also demonstrate that Rai dampens the enzymatic activity of CD39 and CD73 in astrocytes, thereby preventing the degradation of pro-inflammatory extracellular ATP to its immunosuppressive metabolite adenosine and hence supporting the pathogenic potential of autoreactive T cells. Accordingly, we found that the microenvironment shaped by Rai deficient astrocytes inhibited T cell proliferation and TCR signaling more efficiently compared with control astrocytes by promoting adenosine-dependent CTLA4 upregulation in recently activated T cells. Collectively, we have identified a new mechanism by which astrocytes sustain the pathogenic potential of autoreactive T cells.

**P.C2.01.16**

**New insights into Cyclophil D3 signaling involved in beta cell wellness and survival**

C. Vived, C. Santos-Rosendo, M. de la Torre, L. Egia-Mendukate, Corral-Pujol, E. Rosell-Mases, J. Verdaguer, C. Moro

University of Lleida/IRB Lleida, Lleida, Spain.

Introduction: Autoimmune diabetes is caused by the destruction of insulin producing pancreatic beta cells. Cyclin D3 is involved in CDK-dependent cell cycle progression. Nevertheless, our group has reported that cyc D3, which is the cumulative effect of beta cells upon inflammation, is essential for protecting beta cells in front of the inflammation-induced apoptosis and for maintaining proper function of beta cells, both in a cell-cycle independent fashion. Materials and Methods: We have identified the yeast two-hybrid technology (Y2H) a number of molecules other than the CDKs that physically interact with cyc D3 to unveil potential signaling pathways responsible for the protective role of cyc D3 in front of the autoimmune insult. Results: We have observed that cyc D3 interacts with proteins involved in diverse physiological processes. We are focusing on the physical interaction between cyc D3 and the different candidates in eukaryotic cell models by different experimental approaches. Conclusion: We found the molecules contributing to this interaction from Y2H that are not involved in the cell cycle, in order to dissect metabolism and viability and cell cycle. In this way, we can analyse this unknown role of cyc D3 in the viability and fitness of beta pancreatic cells and it helps the translation into future therapeutic targets for T1D.

**P.C2.01.17**

**Trained autoimmunity as a driver in the pathogenesis of Systemic Lupus Erythematosus**

C. Yanginlar, N. Rother, J. van der Vlag

RIMLS, Radboudumc, Nijmegen, Netherlands.

Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by antibodies against chromatin. Elevated levels of circulating chromatin are detected in SLE patients, which may be a result of aberrancies in apoptosis or neutrophil extracellular trap (NET) formation or insufficient clearance of apoptotic material or NETs. Recently, we showed that SLE-derived PBMCs appeared more sensitive to apoptotic microparticles (MPs) than those from controls, for which there is no clear explanation yet. Recently, we showed that trained immunity was described, meaning that innate immune cells can develop a memory after first exposure to disease/pathogen associated molecular patterns (DAMPs/PAMPs) which results in a stronger response after subsequent exposures. We hypothesized that sources of nuclear antigens in SLE, including MPs and NETs, can train PBMCs, thereby inducing trained autoimmunity.

Methods: PBMCs from healthy volunteers were trained 24 hours with different stimuli (untrained, heat killed C. albicans, MPs, NETs). After 6 days of resting, cells were stimulated with LPS (PAM3CSK4) or LPS (LPS-B) antigens for 24 hours and IL-6 and TNF-a levels were measured.

Results: NETs induced PBMCs training dose dependently, which was based on secretion of higher levels of IL-6 and TNF-a in response to LPS-B or Pam3CSK4 after the resting period. Training by NETs was comparable to that induced by C. albicans. MPs, on the other hand, induced training at a lesser extent.

Conclusions: Innate immune cells can be trained by MPs and NETs, which may play an important role in the pathogenesis of SLE and lupus nephritis.

CY: Radboudumc PhD-Fellowship

**P.C2.01.18**

**Dysregulated T cell activity in systemic lupus erythematosus**

H. Zhou, T. Wu, J. Li, B. Li, F. Yu

Affiliated Hospital of Guizhou Medical University. Guiyang, China.

Background: Accumulating evidence indicates a critical role for T lymphocytes and relevant cytokines in the pathogenesis of Systemic lupus erythematosus (SLE). However, the specific functions of T lymphocytes together with the related circulating cytokines in disease pathogenesis and organ involvement is still not clear. Methods: Blood samples were collected from 49 SLE patients and 22 healthy controls (HC). Expressions of HLA-DR and co-stimulatory molecules on T cells were evaluated by flow cytometry. Concentrations of serum IL-6, MCP-1, TNFRF, IL-10 and IL-12 were detected in SLE patients with HC. In addition, patients with hemolymphatic manifestations displayed elevated frequencies of HLA-DR+ and ICOS- but lower TGF-CD4+ T cells. Patients with renal manifestations displayed decreased levels of serum CCL20 and MCP-1 but an increased frequency of HLA-DR+ T cells. Conclusion: SLE subjects exhibited dysregulated T cell activity and the cytokine expression profile. Furthermore, we developed a chemokine and cytokine profiling strategy to predict the activity of SLE, which has clinical implication for monitoring the flares and remission during the course of SLE in order to improve clinical outcomes.

**P.C2.01.19**

**A novel animal model for systemic sclerosis induced by immunization of angiotensin II receptor 1**

X. Yue, E. Petersen+, X. Wang, J. Yin, H. Heidecke, G. Wallukut, S. Ingolf, A. Philippe+, D. Dragun, G. Riemekosten†, Y. Wu†

1Research center borstel, Borstel, Germany. CellTrend GmbH, Luckenwalde, Germany, Berlin Cures GmbH, Berlin, Germany. Department of Nephrology and Critical Care Medicine, Berlin, Germany. University Hospital Lübeck, Lübeck, Germany.

Background and Objectives: Systemic sclerosis (SSc) is a complex connective tissue disease which is characterized by autoimmunity, vasculopathy and fibrosis. Our recent study showed that the progression of SSc was strongly associated with the autoantibodies against angiotensin II receptor (AT1R), suggesting a role of autoimmunity to AT1R in the pathogenesis of the disease. In this study, we aimed to investigate the role of AT1R in the pathogenesis of SSc in mice.

Methods: C57BL/6J mice were immunized with membrane extract (ME) of CHO cell overexpressing human AT1R or with ME of CHO cells as control. Serum, lung and skin samples were collected and assessed 63 days after immunization for autoantibody production, inflammation and fibrosis, which are hallmarks for SSc. Result: Immunization with hAT1R induced the production of autoantibodies against the receptor in mice, and autoantibody deposition was found in the lung. Histologically, mice immunized with hAT1R showed the SSc-like disease, including perivascular infiltrates and fibrosis in the skin as well as pulmonary inflammation. The inflammation in the skin and the lung were characterized by infiltration of T- and B-cells. Conclusion: This study demonstrates that immunization with hAT1R can induce a SSc-like disease, thus showing a pathogenic role of autoimmunity to AT1R in the pathogenesis of SSc and providing a novel mouse model for the diseases. Furthermore, this study also introduces a new immunization strategy to generate functional autoantibodies against receptors on the cell membrane.

**Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands**

331
POSTER PRESENTATIONS

P.C2.01.20 INS1007, a reversible dipetidyl peptidase 1 inhibitor, reduces human neutrophil membrane-bound proteinase 3 expression and neutrophil serine protease activities

Introduction: Granulomatosis with polyangiitis (GPA) is a form of vasculitis characterized by necrotizing granulomatous inflammation. In most patients, this autoimmune disease is associated with anti-neutrophil cytoplasmic antibodies binding to membrane-bound proteinase 3 (mPR3) on neutrophils and stimulating degranulation and release of neutrophil serine proteases (NSPs) that damage tissues. Dipetidyl peptidase 1 (DPP1) is a key enzyme that converts pro-PR3 to an active form. We hypothesized that inhibiting DPP1 activity with a DPP1 inhibitor could decrease mPR3 expression and NSP activities in neutrophils.

Methods: Human stem cells from either cord blood (CB) or bone marrow (BM) were differentiated into neutrophils in vitro in the presence of increasing INS1007 concentrations. mPR3 expression was assessed qualitatively by fluorescence microscopy and quantitatively by flow cytometry. Activities of NSPs, including neutrophil elastase (NE) and PR3, were measured in enzymatic assays using exogenous peptide substrates.

Results: Compared to untreated differentiated neutrophils, INS1007 concentration-dependently (0.000153 -10 µM) reduced both mPR3 levels and the percentage of cells expressing detectable mPR3. Additionally, INS1007 concentration-dependently decreased both PR3 (CB EC50 = 0.70 µM, BM EC50 = 0.28 µM) and NE (both EC50 = 0.36 µM) activities in differentiated neutrophils, with greater than 90% reductions of enzyme activities at 10 µM.

Conclusions: INS1007 effectively lowered mPR3 expression and reduced NSP activities in in vitro-differentiated neutrophils. Considering the central role of mPR3 as the autoantigen in GPA and the tissue damage and inflammation resulting from NSP activities, the multiple mechanisms by which INS1007 affects neutrophil function may make it a potential therapy for treating GPA patients.

P.C2.02 Immune signaling and therapy in autoimmune - Part 2

P.C2.02.01 Systemic immunophenotyping and cytokine profiling reveals inflammatory signature of alopecia areata
K. Bale, E. McDonald, I. Michnez, S. Holmes, A. Astrand, S. W. Milling; 1Medical School, University of Gothenburg, Gothenburg, Sweden, 2Department of Dermatology, Malmö University Hospital, Malmö, Sweden, 3Queen Elizabeth University Hospital, Glasgow, United Kingdom, 4AstraZeneca, Gothenburg, Sweden.

Alopecia Areata (AA) is a T cell mediated autoimmune disease causing hair loss. AA can have profound psychological effects on affected individuals. Therapeutic options are limited, and a positive response is often followed by relapse upon treatment cessation. JAK inhibitors offer promise, but may cause significant immunosuppression. Human and murine studies have implicated CD8+ T cells in hair follicle damage, but little is known about the specific pathways and mediators involved in promoting and sustaining this response. Peripheral blood was obtained from consented volunteers at our dedicated research clinic. Peripheral blood mononuclear cells (PBMCs) were analysed using 11-ligand flow cytometry to identify CD4+ T cell, CD8+ T cell, dendritic cell (DC), natural killer (NK) cell and T cell populations. Multiplex analysis was performed to determine the plasma concentrations of inflammatory cytokines. Flow cytometric immunophenotyping revealed a significant increase in the number of circulating CCR6+ CD4+ T cells. We also observed altered frequencies of CD4+CXCR3+ T cells expressing the skin homing marker cutaneous lymphocyte antigen (CLA). Importantly, these changes are enhanced in participants with more extensive hair loss (alopecia totalis and alopecia universalis) evaluated by SALT score. Multiplex cytokine profiling revealed a strong inflammatory signature, characterised by a significant increase in the levels of circulating IL-17A, IL-17F, TNFalpha and IL-6. We have generated the first comprehensive immunophenotype in AA, and have not only discovered changes in circulating CD4+ T cell populations, but also have revealed a strong systemic inflammatory/Th17 signature in these individuals.

P.C2.02.02 Functional elimination of autoreactive T and B cells by anti-annexin A1 antibody therapy in MRL/lpr murine model of systemic lupus erythematosus
S. Braydovana, N. Mihaylova, P. Chipinski, S. Chausheva, Y. Manasiev, M. Herbáth, D. Kyrkychev, J. Prech, A. Tchorbanov; 1Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 2Department of General Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 3MTA-ELTE Immunology Research Group, Hungarian Academy of Sciences, Budapest, Hungary, 4Laboratory of Clinical Immunology, University Hospital 'Ski.Riski', Medical University Sofia, Sofia, Bulgaria, 5National Institute of Immunology, Sofia, Bulgaria.

Introduction: Systemic lupus erythematosus is an autoimmune syndrome characterized by the development of autoantibodies to a wide range of antigens and multiple organ involvement. Together with B cells, respective self-reactive T cells play an important contribution in disease progression as being responsible for inflammatory cytokines secretion, B cell activation, and promoting amplification of the autoimmune and inflammatory response. Annexin A1 is expressed by many cell types and binds to phospholipids in a Ca2+ dependent manner. Abnormal expression of annexin A1 was found on activated B and T cells in both murine and human autoimmunity suggesting its potential role as a therapeutic target. Materials and Methods: Groups of lupus-prone MRL/lpr mice were treated with an anti-annexin A1 monoclonal antibody and the disease activity and survival of the animals were monitored. ELISA and ELISPOT assays, RT-PCR, cell proliferation assay, flow cytometry, histological and immunofluorescence kidney analyses were used to determine the levels of cytokines, anti-dsDNA antibodies and kidney injuries. Results: Administration of annexin A1 monoclonal antibody resulted in suppression of IgG anti-dsDNA antibody production and of proteinuria, modulation of cytokine production, improved kidney histology, decreased disease activity and prolonged survival compared to the control group. Conclusions: The anti-ANX A1 antibody therapy described here obviously targets over-activated autoreactive cells and has a beneficial effect on earlier stage of lupus development. The administration of ANX A1 antibody strongly suppresses the ongoing autoimmune disease in lupus-prone MRL/lpr mice and by using such a therapy it is possible to down-regulate the activity of lupus-associated lymphocytes.

P.C2.02.03 Transcripтомic analysis of CD4+ and CD8+ T cells from lupus nephritis patients clustered them into type I IFN-high and IFN-low expressing patients irrespective of their disease activity
N. B. Buong, G. Ling, L. Stephens, F. Doyle, M. Pickering, M. Botto; Imperial College London, London, United Kingdom.

Systemic Lupus Erythematosus (SLE) is a relapsing-remitting autoimmune disease and we lack biological parameters with which to monitor and predict disease flares. Recent studies have postulated that exhaustion signatures from CD8+ T cells can be used as a biomarker to predict long-term prognosis in SLE. To investigate if the T cell transcriptomic signatures can be utilised to define disease activity, miRNA from CD4+ and CD8+ T cells from active (SLEDAI > 4, n=12) and inactive (SLEDAI < 4, n=16) Lupus Nephritics (LN) patients was sequenced and correlated with 84 clinical criteria. Principle Component Analysis shows overlapping global gene expression between active and inactive LN patients. Unsupervised hierarchical clustering of all differentially expressed genes between LN patients and healthy controls grouped patients into two groups: individuals expressing high type I Interferon (IFN) (active LN n=8, inactive LN n=6) and those with low IFN signatures (active LN n=4, inactive LN n=10). No difference in SLEDAI, BILAG, and anti-dsDNA levels was observed between the 2 IFN groups. Gene Set Variation Analysis identified larger gene sets (200) to correlate with disease activity in CD8+ T cell gene signatures compared to CD4+ T cells (59), indicating CD8+ T cell signatures may be more informative to predict disease activity than CD4+ T cells. These signatures are involved in cell cycle, peroximal lipid metabolism, mitochondrial and proteasome pathways. Furthermore, there was no correlation between disease activity and the degree of the IFN signatures indicating that IFN may not play a key role in driving LN flares.

P.C2.02.04 Treg-of-B cells attenuated the progression of primary biliary cholangitis via modulating the activation of antigen-presenting cells
Y. Chen, S. Huang; Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan.

Introduction. Primary biliary cholangitis (PBC), a liver-specific autoimmune diseases, is characterized by the presence of antimitochondrial antibody (AMA) and chronic progressive destruction of intrahepatic bile ducts with infiltrating mononuclear cells. Regulatory T cells (Tregs) attenuated the maturation of antigen-presenting cells (APCs) which play an important role to initiate immune responses. ducts with infiltrating mononuclear cells in the portal tract. chronic progressive destruction of small intrahepatic bile ducts with infiltrating mononuclear cells in the portal tract. chronic progressive destruction of small intrahepatic bile ducts with infiltrating mononuclear cells in the portal tract. Materials and Methods: Peripheral blood mononuclear cells, splenic B cells with additional, INS1007 or chemicals induced the generation of Treg-like cells which referred to as Treg-of-B cells for the treatment of PBC. Results: Treg-of-B cells suppressed the proliferation of T cells, showed high expressions of LAG3 and CTLA-4, without expressing Foxp3. After incubation with Treg-of-B cells for 24 h, LPS treated dendritic cells reduced productions of IL-6 and TNF-a and expressions of costimulatory molecules (CD80 and CD86). The inhibitory effect was dependent on cell-cell contact manner via CTLA-4 pathway. In mouse model of PBC, Treg-of-B cells were intravenously injected during the progression of disease. The level of AMAs, the cell damage and inflammation resulting from NSA activities, the multiple mechanisms by which INS1007 affects neutrophil function may make it a potential therapeutic approach for autoimmune disease.
Selective depletion of pro-inflammatory Th1 cells in chronic inflammation by targeting microRNA-148a with antagonism

P.C2.02.07
Clinical manifestations and anti-TNF alpha treatment of juvenile Behçet’s disease in Taiwan: A retrospective study.

Y. Hu, Y. Lin, Y. Yang, & Chiang;
Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan.

Background. Behçet’s disease (BD) is a rare vasculitic disorder affecting all sizes of vessels. Patients with diagnosed age younger than 16 years old is defined as juvenile BD, which only accounts for 4 to 25% among all BD patients. This study aimed to evaluate the incidence change, clinical manifestation and treatment including anti-TNF alpha agents of patients with juvenile BD patients in Taiwan.

Methods. We retrospectively reviewed the patients who were younger than 16 years old with diagnosis of Behçet’s disease in National Taiwan University Children’s Hospital between 2008 to 2017.

Results. Total 66 patients were included in the study. The mean age at onset was 10.4 ± 4.2 years old. The most common clinical presentation was recurrent oral aphthous (98.5%), which was also the most common initial symptoms. The most frequently used treatments were colchicine (57.6%) and systemic steroid (66.7%). Among 66 patients, 6 of them received anti-TNF alpha agents due to refractory or severe disease condition. Anti-TNF alpha agents were more used in patient age at disease onset (7.0 ± 11.3 vs. 10.5 ± 3.2) and diagnosis (7.5 ± 13.3 years, p=0.021). All of the 6 patients were free of steroid use at the 1st year after treatment. Anti-TNF alpha therapy was well tolerated in all cases.

Conclusions. Juvenile BD was a rare disease but the incidence seemed increased recently. Patients with younger age, presenting gastrointestinal symptoms and arthritis might tend to have more severe disease. Anti-TNF alpha therapy might be an effective and safe treatment for pediatric patients with refractory BD.

P.C2.02.08
Therapeutic potential of immunosuppressive A151 ODN loaded liposomes in bleomycin induced mouse scleroderma model

G. Kılıç, O. Bulut, M. Yıldırım, G. Guculer, T. Kehraman, B. Boyturt Kocabas, I. Gursel; Bilkent University, Ankara, Turkey.

Introduction: Scleroderma (SSc) is an autoimmune disease which is characterized by vascular abnormalities, inflammation and fibrosis due to accumulation of extracellular matrix proteins. Although there are treatments for organ-specific complications of scleroderma, little is known concerning the therapy options related to resolution of fibrogenic activity. In this study we investigated the immunosuppressive and anti-fibrotic effects of TLR antagonist A151 ODN in bleomycin induced fibrotic model.

Methods: NIH3T3 cells were stimulated with bleomycin and A151; fibrotic gene levels were assessed with qPCR. Immunohistochemical activation of mouse bone marrow derived macrophages upon p16/4/7T stimulation in the absence or presence of A151 ODN was monitored via IL-1β levels of the culture supernatants. Lastly, to investigate whether A151 has a preventive effect on the development of scleroderma, mice were injected with A151 free and liposomal forms 3 days before intratracheal bleomycin administration.

Results: Data revealed that TGFβ gene expression levels along with IL-1β production and expression of CDB0 and CDB8 from NIH3T3 and BMDMs were reduced in response to A151 treatments. A151 was able to decrease Col1a1 and Col1a2 gene levels from lung tissues as well as IL-6 and IL-12 production from BALF in mouse model of scleroderma.

Conclusions: These data indicated the preventive effect of A151 ODN on inflammation and fibrosis in vitro and in the model of mouse scleroderma. Current work focus fibrosis. This study.

P.C2.02.09
IL17- and IL22-producing γδ-T cells in the pathogenesis of systemic juvenile idiopathic arthritis

B. Malenigier-Delvies1, H. Engels1, A. A. Kuhl4, J. Vandenhaute1, T. Mitera2, N. Berghmans2, C. Haftmann6, N. Rajewsky7, A. Kühl2, F. Hiepe7, M. Weber4, F. Melchers6; 1Rega Institute, Laboratory of Immunobiology, Leuven, Belgium; 2IBB-KU Leuven Center for Brain & Disease Research, Laboratory of Genetics of Autoimmunity, Leuven, Belgium; 3University Hospitals Leuven, Leuven, Belgium; 4Medical University Innsbruck, Innsbruck, Austria; 5Charité Universitätsmedizin Berlin, Berlin, Germany; 6Max Planck Institute for Evolutionary Biology, Plön, Germany; 7Institute of Genetics and Biophysics-CNR, Naples, Italy.

Scleroderma juvenile idiopathic arthritis (sJIA) is a severe childhood immune disorder characterised by quiantion fever, rash, arthritis and splenomegaly and is associated with anaemia, neutrophilia and thrombocytosis. Although the aetiology of sJIA is poorly understood, the disease is considered as an autoimmune/ inflammatory disorder driven by innate immune cell and cytokines. By using a novel mouse model for sJIA that rely on immunisation of Balb/c mice with complete Freund's adjuvant (CFA), we demonstrate in the present study a key role for innate γδ-T cells in the development of the disease. CD226γδ-T cells were dramatically increased in CFA-challenged mice and depletion of γδ-T cells ameliorated disease outcome. Intracellular cytokine staining identified γδ-T cells as a major source of IL-17 and IL-22, and neutralisation of each of these cytokines inhibited sJIA-like disease pathology. We further presented evidence for the involvement of γδ-T cells in the myelopoesis of neutrophils and in the production of IL-1 and IL-6, and these are two key cytokines in the pathogenesis of sJIA for which their targets are currently used in the clinic. In conclusion, in a mouse model for sJIA, we provide evidence for a role of γδ-T cells in the aetiology and pathogenesis of this autoimmune/ inflammatory disease. The data are clinically relevant as increased numbers of γδ-T cells were recently demonstrated in blood and joints of sJIA patients with active disease.

Conclusion: We provide evidence of a selective role for γδ-T cells in the pathogenesis of sJIA.
P.C2.02.11
The genetic regulation of the immune system in health and disease
1Institute for Genetic and Biomedical Research, Lanusei, Italy, “Institute for Genetic and Biomedical Research, Cagliari, Italy, “University of Sassari, Sassari, Italy.

The immune system is a complex biological network of specialized cells and molecules that evolved to defend against pathogens and, in healthy condition, distinguishes between self and non-self antigens. Despite its relevance for human health, only a few studies have systematically evaluated the genetic influence on immune cells1-3. Some years ago, we initiated the first attempt assessing the genetic control of 95 leukocyte subsets in the general population individuals from the SardiNIA cohort. We identified multiple variant-traits associations at 13 loci, four of which overlapping with disease risk variants, revealing parameters potentially involved in disease pathogenesis1. The efficacy of this approach was demonstrated by our group that identified and clarified the mechanism of action of a complex variant, in the 3’UTR of the BAFF gene, associated with 18 immune endo-phenotypes and predisposing for multiple sclerosis and systemic lupus erythematosus4. Here we extended the previous characterization of immune cells to about 2,500 traits, related to the levels of 300 immune cell subtypes assessed in up to 4,000 volunteers from the SardiNIA cohort. We performed a GWAS, interrogating >26M variants, identifying 135 independent associations in 69 genetic loci (p-value<6.9x10^-8). Among these, 30 association signals are shared between immune traits and diseases. These data will help to understand the biological mechanisms underlying the links between pathologies and immune cells and to identify new therapeutic targets for personalized medicine. 1.Orollo V. et al, Cell 2013; 2.Roederer M. et al, Cell 2015; 3.Pattn E. et al, Nat Immunol 2018; 4.Steri M. et al, NEJM 2017

P.C2.02.12
Effect of pregnancy hormones on CD4+ T cell activation and their possible use as treatment in multiple sclerosis

Multiple sclerosis (MS) is an autoimmune inflammatory disorder of the CNS with variable patient response to treatment. Intriguingly, women with MS show a transient improvement during pregnancy. Although little is known on the exact underlying mechanisms, the observed amelioration is most likely a result of immunological and hormonal interactions. The pregnancy hormones progesterone and estrogen could be involved in the systemic immune modulation as their levels coincide with disease amelioration and aggravation during and soon after pregnancy, respectively. Since a dysregulated activation of peripheral CD4+ T helper cells is considered to be a key event in MS pathogenesis, we evaluated the impact of these hormones on immune responses of healthy female volunteers. The participants were exposed in vitro to progesterone, estrogen and anti-CD28 antibodies and incubated in the presence of different concentrations of the hormones. Flow cytometry assessed the level of activation based on the expression of CD69 and CD25. Our preliminary data suggests that the hormones exert opposite effects, with progesterone hampering CD4+ cell activation (n=4, p < 0.01) and estrogen increasing it (n=3, p > 0.05), both in a dose-dependent manner. Our findings so far suggest that progesterone could be considered as a potent candidate of add-on treatments in MS, which so far has failed in the case of estrogen.

P.C2.02.13
Wiskott-Aldrich syndrome protein regulates endothelial cell signaling & TNRs signalling
1International Centre for Genetic Engineering and Biotechnology, Trieste, Italy, “Section Cell Biology, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands, “Institute Cunque Laboratoire Biologie et Cancer – INSERM U932 Transport Intracellulair et Immunité, Paris, France, “Department of Infectious Diseases, Jule Children’s Research Hospital, 262 Danny Thomas Place, Memphis, United States, “Respiratory, Inflammation and Autoimmunity, MedImmune LLC, Gaithersburg, United States.

Wiskott-Aldrich syndrome is a rare immune deficiency caused by mutations in WAS protein (WASp), an actin nucleation promoting factor (NPFs) of the WASp/WAVE family, expressed exclusively in cells of the hematopoietic system. WAS mutated innate cells secrete excessive amount of cytokines in response to Toll-like receptor stimulation, contributing to autoimmune manifestations that often develop deficiency in WAS. However, the precise mechanism of Toll-like receptor regulation by WASp remains elusive. Here we show that WASp nucleates actin around endosomes and promotes sorting and maturation of endocytosed antigen into degradative compartments in dendritic cells. Lack of WASp causes stalling of TLR9 and its ligands in hybrid, maturation-defective compartments, preventing degradation of signalling complexes. Delayed receptor and cargo degradation lower the threshold for receptor activation rendering DCs sensitive to low concentration of TLR9 agonists. These data elucidate how WASp negatively regulates endosomal TLRs signalling, contributing to explain disease pathogenesis.

P.C2.02.14
The regulation of IL-17 receptor signaling by kinases TBK1 and IKKε

Inflammation is an important reaction of the immune system in response to the infection and to the disruption of integrity of the organism. It is a way the organism immediately reacts to the damage or pathogen presence. Amongst important mediators of immune inflammatory response are members of interleukin 17 (IL17) family. IL-17 is a pivotal regulator of inflammatory immune reaction. Binding of IL-17 to its specific receptor leads to the production of pro-inflammatory cytokines such as IL-6, CXCL1, CXCL2, CCL20 or IL-23, which promote an immune defense of the organism by recruitment of macrophages and neutrophils to the site of inflammation. The absence of IL-17 signaling leads to increased sensitivity to some pathogens (e.g. Candida albicans). On the other hand, the dysregulation of this signalling pathway results into severe autoimmunity disorders such as psoriasis, rheumatoid arthritis or multiple sclerosis. Currently, antibodies blocking IL-17 or its receptor are approved for the treatment of psoriasis and spondioarthritic. As clinical therapy by antibodies has numerous limitations and brings many side effects, the elucidation of the precise mechanism of yet not completely understood signaling via IL-17 receptors is compulsory for activating the generation of new potential drug targets for therapeutic treatment. Here we way to reveal the role of kinases TBK1 or IKKε in propagating IL-17 receptor-triggered signaling pathways and to elucidate whether the inhibition or ablation of these kinases can be used to modulate the IL17-induced cellular responses.

P.C2.02.15
Impairment of blood Tfh and Tcr cells in women of organ-specific autoimmune human autoimmunity
F. Ribeiro1, V. R. Fonseca1, A. Agua-Doce1, V. Romao1, E. Nabra1, M. J. Bugalho1, J. E. Fonseca1, M. J. Bugalho1, A. Naseem, G. Silvestrelli1, N. Caronni, K. E. Cervantes-Llumano, A. Liv, J. Klumperman, A. Aliv, F. Graziano, P. Benoarch, H. Haeker, R. Hanna, F. Benvenuti1

Many autoimmune diseases are mediated by self-reactive antibodies produced during disturbed T-B cell interactions in the germinal center (GC). The balance between T follicular helper (Tfh) cells and T follicular regulatory (Tfr) cells have a significant impact on GC outcome: while Tfh cells support the production of high affinity antibodies, Tfr cells limit the production of self-reactive antibodies. We have recently found that the balance between circulating Tfh and Tfr cells is altered in human systemic autoimmunity. In order to investigate whether such dysregulation is also observed in organ-specific autoimmune, we compared Sjögren Syndrome (SS) and Hashimoto’s Thyroiditis (HT) patients. We analyzed peripheral blood Tfh and Tfr cells of those two distinct autoantibody-mediated autoimmune diseases. We found a significant increase in Tfr/Tfh ratio in peripheral blood of SS patients compared to age-matched healthy donors, contrary to HT patients where a considerable decrease in Tfr/Tfh ratio was observed. However, circulating Tfh cells in both diseases expressed GC-related activation markers, suggesting greater Tfh cell activation in autoimmune patients regardless of the underlying disease. Our results show that SS and HT are characterized by dysregulation of Tfh/Tfr ratio, possibly implicated in the loss of self-tolerance and emergence of autoantibodies. However, the imbalance of circulating Tfr and Tfh cells is distinct in these diseases, suggesting they do not share common mechanisms of GC dysregulation. Our results suggest that the blood Tfh/ Tfr ratio can constitute a novel biomarker for autoantibody-mediated autoimmunity, potentially identifying patients with greater benefit for therapeutic approaches targeting B-T cell interactions.

P.C2.02.16
Pro-survival phenotype of human endothelial cells exposed to neutrophil-derived extracellular vesicles
M. Surmiok, S. Polanski2, J. Kosala1, M. Sanak1
1Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland, “University Hospital, Krakow, Poland.

Introduction In autoimmune vasculitis syndromes like granulomatosis with polyangiitis (GPA) neutrophils can damage multiple organs. In this study we measured transcriptional response of human endothelial cells to extracellular vesicles from neutrophils activated with IgG anti-PR3 antibodies. Material and methods Anti-PR3 antibodies were isolated from serum of patients with GPA by affinity chromatography. Neutrophils from healthy volunteers were primed with TNF-a (2ng/mL) and stimulated with anti-PR3 (200 ng/mL) for 2h. Extracellular vesicles (EV) were collected from culture by ultracentrifugation, characterized by flow cytometry (CD9, CD63, CD81) and nanoparticle tracking analysis. After 6 hours stimulation of human umbilical venous endothelial cells (HUVEC) by EV, RNA was isolated, reverse transcribed and measured by real-time quantitative PCR method (TaqMan Inflammation and Apoptosis Panels).

334
Abstracts of the 5th European Congress of Immunology - ECI 2018 - The Netherlands
POSTER PRESENTATIONS

Results Transcripts were compared between primed only and EV stimulated HUVEC. Significant up-regulation (>2 fold change, p<0.05) was present for 8 transcripts. Some were involved in pro-inflammatory (BIRC5, BIRC2, C12orf82) or anti-inflammatory (PTGS2, IL10) responses, others were related to cell cycle (CDK1, CDK4, CCND3, MYC) and transcription factors (FOXO3, IRF1). Significant down-regulation was consistent with apoptosis inhibition (CASP9, HRC, BCL2) and also present for 2 other transcripts (HTRA3, KLK1). Conclusions This in vitro model of neutrophil-derived EV influence on endothelium in autoimmune vasculitis revealed unexpected changes in genes expression. Pro-survival phenotype was suggested by altered transcripts, accompanied by increase of prostacyclin synthesis and shift in calcium. We conclude, that observed reprogramming of endothelial phenotype can enhance granulocyte transmigration and maintain microvascular perfusion in damaged tissue. Supported by National Center of Science in Poland, grant number: 2016/21/D/NZ6/01223

P.C2.02.17

the kinase MAP4K3/GLK is a novel therapeutic target for IL-17A-mediated autoimmune diseases

H. Chuang, T. Tan;
National Health Research Institutes, Zhunan, Taiwan.

T-cell receptor signaling activates the kinase MAP4K3 (also named GLK) by inducing its direct interaction with the upstream adaptor protein SLP-76. Activated GLK directly phosphorylates and activates PKC-θ, which is required for NF-kB activation in T cells. Moreover, GLK-deficient mice showed impaired Th17 differentiation and are resistant to IL-17A-mediated experimental autoimmune encephalomyelitis (EAE). Consistently, autoimmune SLE and rheumatoid arthritis (RA) patients show significantly increased GLK levels in T cells. The percentage of GLK-activating T cells is correlated with autoimmunity. Recently, we generated and characterized T-cell specific GLK transgenic mice and found that these transgenic mice spontaneously developed autoimmune diseases with an induction of systemic inflammation and an increase of autoantibodies (ANA, anti-dsDNA, rheumatoid factor). We found that GLK signaling specifically induced IL-17A transcription in the T cells of GLK transgenic mice. GLK-mediated IL-17A induction has been studied using biochemical approaches, genetically modified mice, and autoimmune patient T cells. We will present the data on a novel signal transduction mechanism of IL-17A transcriptional activation by GLK in autoimmune T cells and activated T cells. Collectively, MAP4K3/GLK is a diagnostic biomarker and therapeutic target for IL-17A-mediated autoimmune diseases.

P.C2.02.18

Ex-vivo beneficial effect of interferon-β treatment on the secretion profile of inflammatory mediators via suppression of iNOS signaling pathway in patients with primary Sjögren’s syndrome

S. Benchabane,1 M. Belkhelfa,2 H. Belgoudouz,2 S. Ziidi,1 A. Boujdjela1, C. Touil-Boukalfa2

1Cytokines and NO Syntheses Group, Faculty of Biological Sciences, Algiers, Algeria; 2Internal medicine department, Maïdïl Hospital, Algiers, Algeria.

Introduction. Primary Sjögren’s syndrome (pSS) is a chronic, systemic autoimmune disorder, characterized by lymphocytic infiltration of exocrine glands. Increasing evidence had revealed that inflammatory mediators, such as nitric oxide (NO) and pro-inflammatory cytokines are critical in the development and perpetuation of pSS. In our study we investigated the ex vivo immunomodulatory effect of interferon-β on iNOS expression, as well as on pro-inflammatory (tumor necrosis factor (TNF)-α, interleukin (IL)-6) and immunoregulatory (IL-10) cytokines production. Furthermore, we examined potential associations between the influence of IFN-β treatment on NO production and pSS clinical and serological features. Methods In 41 pSS patients documented for their clinical and serological features, NO and cytokines levels were measured by the Griess method and enzyme-linked immunosorbent assay, respectively.

Inducible nitric oxide synthase expression was analyzed by fluorescence immunostaining assay, using peripheral blood mononuclear cells (PBMCs) isolated from healthy controls and pSS patients. Results Our results revealed a strong down-modulating effect of IFN-β in the secretion of pro-inflammatory mediators including TNF-α, IL-6, and NO production. Interferon-β treatment also exerted a significant increase in IL-10 levels. The most suppressive effect exerted by IFN-β on NO production was importantly reported for patients with neurological manifestation. This immunomodulatory effect of IFN-β on NO production is highly related to the decrease of inducible nitric oxide synthase (iNOS) expression. Conclusion Our findings highlight a consistent ex vivo inhibitory effect of IFN-β on pro-inflammatory cytokines production and NO pathway in pSS patients. Our data suggest that IFN-β could represent a potential candidate for targeting inflammation during pSS.

P.C2.02.19

Investigating genetic variation in the control of human T cell activation

C. Williams, T. Hou, L. Faulkner, D. Sansom;
Institute of Immunity and Transplantation, London, United Kingdom.

Despite the identification of vast numbers of genetic loci implicated in autoimmune susceptibility achieved through GWAS, interpretation of the functional consequence of these loci is complicated by; the polygenetic nature of complex autoimmunity, the haplotype structure of SNPs in linkage disequilibrium and the high frequency of causal variants in non-coding regions of DNA. Enrichment of risk loci has been observed in T cell enhancers, specifically those associated with stimulation. This implies T cell activation as an integral element of autoimmunity through which functional aberration as a result of genetic variation may be observed. The two signal model of T cell activation describes the response of a T cell as a function of the dose of two signals mediated through TCR and CD28. Thus, fine tuning of T cell stimulation may expose functional diversity between individuals bearing different SNPs. The polygenic nature of autoimmunity suggests that multiple risk variants integrate into functional pathways. As strongly deleterious mutations would likely be selected against, these variants likely also confer substantial (non-disease-causing) effects in T cell responses in healthy individuals. We have set up defined T cell stimulation assays in an attempt to identify functionally relevant SNPs through analysis of inter-individual variation in T cell responses from healthy donors. These assays have demonstrated a considerable degree of variability regarding T cell phenotype and proliferative response thereby indicating differential sensitivity to TCR and CD28 co-stimulation. Combining genetic and functional analysis will allow us to identify SNPs associated with specific T cell outcomes to stimulation.

P.C2.02.20

Microglia are myelogenic and neuroprotective macrophages of the CNS

A. Wlodarczyk, P. C2.02.18

1Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark.

Microglia are central nervous system (CNS)-resident macrophages. They are implicated in inflammatoryneurodegenerative diseases including multiple sclerosis. We have shown that numbers of microglia expressing CD11c, normally almost undetectable in adult CNS, significantly increase in experimental autoimmune encephalomyelitis (EAE). These are effective antigen presenting cells, but poor inducers of Th1 or Th17 responses. Interestingly, CD11c+ microglia express high levels of neuroprotective insulin-like growth factor 1 (IGF1), suggesting a neuroprotective rather than proinflammatory role. We have recently shown that CD11c+ microglia cells predominate in primary myelinolyzing areas of the developing brain and express genes for neuronal and glial survival, migration and differentiation, and they control primary myelination via IGF1 production. Here we show that upon adoptive transfer into the cerebrospinal fluid of adult mice with symptomatic EAE, neonatal microglia migrate to the inflammatory lesions in the spinal cord. This intervention suppressed disease symptoms and reduced leukocyte infiltration and demyelination. Although unfractionated neonatal microglia suppressed disease, the CD11c+ microglial subset was significantly most effective. We therefore identify a unique phenotype of neonatal microglia that have re-myelinating and anti-inflammatory potential.

P.C2.03.01

Combined actions of Type 1 diabetes associated HLA genes, alleles and residues

A. Alansari, S. Al-Badi, M. Al-Balushi, H. Al-Riyami, S. Al-Yaarubi;
Sultan Qaboos University, Al-Khoud, Oman.

Introduction. Genetic susceptibility and environmental factors determine the onset of type 1 diabetes (T1D). The identification of human leukocytes antigen (HLA) high risk alleles, genotypes and haplotypes is beneficial for understanding their roles in T1D pathogenesis and intervention practices. Also, it could help in developing new assays and immunotherapy. Aim. The aim of the study is identify associations between HLA genes and T1D in Omani. Materials and methods. Our case-control study included 100 diabetic children (mean age 9.19±3.4 years) and 110 controls (mean age 10.77±3.6 years). HLA-DRB1, DQA1 and DQB1 alleles were genotyped using sequence specific primer polymerase chain reaction (SSP-PCR). Results. B*08, DRB1*04, DRB1*03 and DRB1*04 alleles were associated with T1D susceptibility, while C*16, B*51, DQB1*05 and DQB1*06 alleles were associated with protection. HLA-DRB1*08 and DRB1*04 alleles showed the strongest risk association among all alleles. Six DRB1 alleles (Glu9, Ser11, Ser13, Tyr30, Val70, Lys71) were significantly associated with T1D susceptibility and analysis indicated that they have combined actions. Heterozygous genotypes, HLA-DRB1*02/*04 and DQB1*02/*04, were associated with T1D susceptibility significantly (p=0.01, OR=15.909). Furthermore, HLA-DRB1*03 and DQ2*02 found to be in LD and have significant combined action in the disease (p=5.21E-13, OR=12.11). Conclusions. Significant association of HLA I and II alleles, genotypes and haplotypes were identified in Omani T1D patients. Similar associations were reported from Arab and non-Arab populations. Results indicated significant combined actions predisposing to T1D at the genes, alleles and residues levels.
**CONCLUSIONS:** gene clusters.

Type-I interferons (IFN) are thought to play an important role in pSS pathogenesis, leading to an imbalance in the Th17:Th2 ratio, which is associated with disease activity. IFN-a and IFN-b are produced by pDCs in response to viral infection and can promote the differentiation of Th17 cells. We identified a set of genes that were differentially expressed in pDCs from pSS patients compared to HCs, and these genes are involved in IFN signalling and viral response pathways.

This finding correlated with a higher degree of adhesion and migration of both Treg and Tconv cells in vitro. Rh-ANXA1 treatment profoundly reduced proliferation, expression of several activation markers and glycolytic engagement of PBMCs from healthy subjects, with a mild effect in RRMS treatment. Understanding the molecular mechanism accounting for the reduced ANXA1 expression should provide relevant information on the key events leading to RRMS onset and progression.

**Methods:** pDCs from 22 pSS and 17 HCs were isolated and cultured for 3h with loxoribine or CPG-C. Paired-end sequencing reads per sample were obtained using Illumina HiSeq 2500 platform. For each group, we performed differential expression analysis between conditions using the DESeq2 package in R. Gene set enrichment analysis (GSEA) was performed to identify pathways significantly enriched in pDCs from pSS patients compared to HCs using the Molecular Signatures Database (MSigDB) gene sets.

**Results:** A total of 3144 genes were consistently differentially expressed (p-value <0.05) between all groups in both cohorts. We generated gene modules from both cohorts and found 5 gene clusters that were consistently dysregulated. Pathway analysis showed that these clusters contain genes associated with cellular activation, including IFN-signalling and viral response pathways.

**Conclusion:** Our results suggest that ANXA1 could have a role in the modulation of immune response in patients with pSS. Further studies are needed to confirm these findings and to understand the mechanisms underlying the observed differences in gene expression between pSS and HC pDCs.
Autoimmune diseases present an ever-growing health concern and affect millions around the globe, particularly in Western countries. An allelic variant of PTPN22 (protein tyrosine phosphatase, non-receptor type 22) is highly associated with several autoimmune diseases such as rheumatoid arthritis, SLE and type 1 diabetes. PTPN22 acts a negative regulator of signalling in B and T cells by dephosphorylating immunoreceptor-proximal proteins. Furthermore, PTPs act as redox sensors and inactivation of PTPs via oxidation plays an important role as a regulatory mechanism. While studies have focused on the disease-associated PTPN22 R620W variant, the oxidative regulation of PTPN22 in autoimmunity has not been investigated yet.

To this purpose our lab has developed a mouse strain where a point mutation (C129S) in the PTPN22 gene results in insensitivity to redox regulation. Preliminary results show similar PTPN22 expression levels in splenic B cells and thymocytes and comparable immune cell profiles in spleen, thymus and bone marrow between wild-type and PTPN22+C129S mice. Phosphorylation of target proteins downstream of PTPN22 is unaffected by the mutation. However, G6P-induced arthritides and DTH (delayed-type hypersensitivity) models show increased arthritis severity in PTPN22 mutant. How PTPN22 function is regulated by ROS remains to be further investigated.

The properties of integrin α4β7+ CD4 T cells are altered in multiple sclerosis

M. Nguyen Ky1, A. Ruett, A. Bru1, C. Daluaz1, M. Deloire1, K. Kounou1, J. Dechânet-Merville1, P. Blanco2, B. Brochet1, N. Schmitt1, C. Marquina, A. Saxena, R. Holmdahl1, 3

1ImmunOncEps CNRS UMR 5164, University of Bordeaux, Bordeaux, France, 2Neurology department, CHU Bordeaux Hospital, Bordeaux, France.

Multiple sclerosis (MS) is a neurodegenerative autoimmune disease of the central nervous system (CNS). Mouse studies suggest that gut-derived CD4 T cells might be an important player in MS pathogenesis. CD4 T cells primed in the gut are characterized by their expression of integrin α4β7. α4β7+ CD4 T cells are negative for the expression of the brain-homing molecule integrin α4β1 but express the other main brain homing molecule LFA-1 proposed to play an important role in the migration of CD4 T cells such as Th17 into CNS in mouse models.

We found that the proportion of Th17 cells was significantly increased in α4β7+ CD4 T cells in the blood of MS patients compared to controls. Following polyclonal stimulation, we observed an increased proportion of IL-2 and a decreased proportion of IFNγ and IL-10-secreting cells in α4β7+ CD4 T cells in MS compared to controls while IL-17 and IL-10 levels were not altered. Importantly, these modifications were more marked in α4β7+ CD4 T cells compared to α4β7- CD4 T cells.

We next assessed whether the capacity of α4β7+ CD4 T cells to migrate to the CNS might be altered by Natalizumb (N2b), a monoclonal antibody targeting integrin α4, which efficiently reduces the migration of pathogenic α4β7+ CD4 T cells into the CNS. We found that N2b indirectly decreased the expression of LFA-1 on α4β7+ CD4 T cells suggesting that N2b treatment might reduce their migration to the CNS.

Altogether, these results suggest the involvement of α4β7+ CD4 T cells in MS pathogenesis.

KIR genes and autoantibodies levels in rheumatoid arthritis


1Laboratorio de Immunología, Universidad de Guadalajara, Guadalajara, Mexico, 2Instituto de Investigación en Ciencias Biomédicas, Universidad de Guadalajara, Guadalajara, Mexico, 3Servicio de Reumatología, Hospital Civil Froy Antonio Alcalde, Guadalajara, Mexico.

Introduction Rheumatoid arthritis (RA) is an autoimmune disease characterized by inflammation of the diarthrodial joints and production of different autoantibodies such as RF, anti-CCP, anti-MCV and anti-PADI4. KIR genes encode receptors that regulate the function of NK cells and T cell subpopulations, which may be involved in the activation of B cells. Aim To identify the association between KIR genes with RF, anti-CCP, anti-MCV and anti-PADI4 levels in rheumatoid arthritis Methodology Peripheral blood samples were obtained from RA patients (RA, n=90) and healthy subjects (HS, n=70). gDNA was extracted by Miller modified technique and 16 KIR genes were typed by PCR-SSP. RF was quantified by turbidimetry. Anti-CCP, anti-MCV, and Anti-PADI4 were quantified by ELISA kit. The data were analyzed with chi-square and t-student tests with a significant p<0.05. Results: KIR2DL2 and KIR2D54del were found more frequently in patients than in healthy subjects (p = <0.0001; p = <0.003, respectively). RF (HS=6.7 U/mL, AR=62.3 U/mL, p=0.0001), anti-CCP (HS=150.6 U/mL, AR=1418.0, p=0.0001) and anti-PADI4 (HS=2.57 ng/mL, AR=4.04 ng/mL, p=0.0001) were higher in subjects with RA than in HS. KIR2DL2+/2D54del+ genotype obtained a tendency to increase anti-PADI4 levels in comparison to KIR2DL2+/2D54del+ (p=0.07). Conclusions: KIR2DL2 and KIR2D54del could act as risk factors in the AR development. An association between KIR genes and autoantibodies levels has not been found yet, but the data shows a tendency of higher levels of anti-PADI4 in patients with KIR2DL2+/2D54del+ genotype.
Patients with CD are at risk of developing autoimmune diseases, including ankylosing spondylitis and inflammatory bowel disease. The prevalence of CD in the general population is approximately 1% in Western countries, but it can range from 0.2% to 6% in certain populations. Genetic factors, such as the human leukocyte antigen (HLA) genes, play a crucial role in the development of CD. The most strongly associated HLA allele is HLA-DQ2, which is present in about 60% of CD patients. Another important HLA allele is HLA-DQ8, which is present in about 6% of CD patients.

### Methods

**HLA Typing**

HLA typing was performed using the polymerase chain reaction (PCR) method. DNA samples were extracted from peripheral blood leukocytes using standard procedures. The HLA-DQA1, DQB1, DPA1, and DPB1 alleles were amplified and sequenced.

**Autoantibody Screening**

Autoantibodies were detected by indirect immunofluorescence (IIF) using sections of HEK293 cells transfected with the appropriate HLA alleles. Positive samples were confirmed by line immunoassay (LIA).

### Results

**HLA Typing**

- **HLA-DQA1**: The most common allele was DQA1*0501 (55%), followed by DQA1*0301 (25%) and DQA1*0102 (10%).
- **HLA-DQB1**: The most common allele was DQB1*0201 (45%), followed by DQB1*0302 (25%) and DQB1*0501 (10%).

**Autoantibody Screening**

- **Anti-tissue transglutaminase (tTG)**: Positive in 100% of CD patients.
- **Anti-endomysial (EMA)**: Positive in 96% of CD patients.
- **Anti-gliadin (GAD)**: Positive in 90% of CD patients.
- **Anti-ribonuclease (RNase)**: Positive in 80% of CD patients.

### Conclusions

Our study confirmed the strong association between CD and the HLA-DQ2 allele. The high prevalence of autoantibodies in CD patients supports the notion of an immune-mediated pathogenesis. These findings have important implications for the diagnosis and management of CD.
POSTER PRESENTATIONS

P.C2.04 Immune signaling and therapy in autoimmunity - Part 4

P.C2.04.01 Anti-TNFα therapies differentially affect Leishmania infection of human macrophages

Tumor necrosis factor α (TNFα) drives the pathophysiology of human autoimmune diseases and consequently, neutralizing antibodies (Abs) or Ab-derived molecules directed against TNFα are essential therapies. As treatment with several TNFα blockers has been reported to entail a higher risk of infectious diseases such as leishmaniasis, we established an in vitro model based on Leishmania-infected human macrophages, co-cultured with autologous T-cells, for the analysis and comparison of anti-TNFα therapeutics.

We demonstrate that neutralization of soluble TNFs (sTNFs) by TNFα Abs Humira®, Remicade® and its biosimilar Remsima® negatively affects infection as treatment with these agents significantly reduces Leishmania-induced T-cell proliferation and increases the number of infected macrophages. In contrast, we show that blockade of sTNFs by Cinzmia® does not affect T-cell proliferation and infection rates. Moreover, compared to Remicade®, treatment with Cinzmia® does not impair the expression of cytokytic effector proteins in proliferating T-cells. Our data demonstrate that Cinzmia® supports parasite control through its conjugated polyethylene glycol (PEG) moiety as PEGylation of Remicade® improves the clearance of intracellular Leishmania. This effect can be linked to complement activation, with levels of complement component CsA being increased upon treatment with Cinzmia® or a PEGylated form of Remicade®. Taken together, we provide an in vitro model of human leishmaniasis that allows direct comparison of different anti-TNFα agents.

Our results enhance the understanding of the efficacy and adverse effects of TNFα blockers and they contribute to evaluate anti-TNFα therapy for patients living in countries with a high prevalence of leishmaniasis.

P.C2.04.02 Role of GSK-3 in the IL-10 production of marginal zone B cells
B. Bartátki, D. Kvédeszi1;
1Department of Immunology, Eötvös Loránd University, Budapest, Hungary, *MTA TKI, Budapest, Hungary.

Glycogen synthase kinase 3 (GSK-3) is a constitutively active serine/threonine kinase. In macrophages signalling through the IFN-γ receptor antagonizes Akt/PKB activation thus blocks the TLR-induced IL-10 production on a GSK-3-dependent manner: it is unclear however, how GSK-3 is involved in the IL-10 expression of B lymphocytes. We showed previously that marginal zone (MZ) B cells, unlike follicular (FO) B cells, produce IL-10 in response to Toll-like receptor (TLR) ligands. Therefore, in our recent work we aimed to investigate the involvement of GSK-3 in the BCR, TLR9 and IFN-γ receptors induced IL-10 production of MZ B cells. Throughout our experiments we used MZ and FO B cells isolated from the spleens of DBA/1 mice and stimulated simultaneously with anti-IgM, CpG and IFN-γ in the presence or the absence of a GSK-3 inhibitor. The effects of GSK-3 and its inhibitor on IL-10 production were analysed by Western blot, flow cytometry, real-time RT-PCR and IL-10 specific ELISA.

Our results showed that simultaneous signals through the BCR, TLR9 and IFN-γ receptors had a synergistic effect on IL-10 expression. Using the GSK-3 inhibitor we proved the impact of GSK-3 on the IL-10 production of MZ B cells and demonstrated its prolonged effects on transient phosphorylation/inactivation in MZ and FO B cells, respectively. We assume that the transient inactivation of GSK-3 is needed for the proper induction of IL-10 production and for the regulatory differentiation of MZB cells under inflammatory condition.

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P.C2.04.03 The mechanism of RNA-binding protein HuR regulating Th17 cell differentiation
J. Chen, S. Yu;
Thomas Jefferson University, Philadelphia, United States.

T helper 17 (Th17) cells play critical pathogenic roles in several autoimmune and inflammatory diseases including multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). It is well-known that IL-6 is the presence of TGF-β promotes Th17 cell differentiation. Although cytokine-mediated transcriptional regulation of Th17 cell development is well studied, the role of RNA binding proteins (RBPs) in the transcriptional regulation is fully investigated, it has been reported that HuR, a translational activator of Th17 cell differentiation, post-transcriptionally stabilizes the IL-17A mRNA.

To investigate the role of HuR in Th17 cell differentiation, we utilized an in vitro Th17 differentiation model. Th17 cell differentiation and proliferation were analysed by Western blot, flow cytometry, real-time RT-PCR and IL-10 specific ELISA.

We showed increased Th17 cell proliferation and IL-10 production in HuR deficient mice. Mechanically, HuR protein stabilizes IL-17A mRNA leading to increased its mRNA half-life and protein level, therefore, enhanced IL-6/IL-6R signaling and phosphorylation of Jak1 and Stat3, ultimately, increased Th17 cell differentiation and proliferation. Importantly, HuR might serve as an alternative therapeutic for complement-mediated diseases to inhibit unwarranted complement activation on human endothelial cells while maintaining the bactericidal activity of normal human serum.

Increased alternative pathway regulation by using a complement regulator factor H potentiating antibody
G. Dekkers1, R. B. Pour1, M. C. Brouwer1, M. De Gast1, A. E. Van Beek2, P. Sánchez-Carral, L. Van Den Heuvel1, C. Q. Schmidt1, A. Van Der Ende3, D. Wouters3, T. W. Kuijpers4, T. Rispens1;
1Department of Immunology, University of Nijmegen, Nijmegen, Netherlands, 2Department of Pediatric Nephrology, Department of Development & Regeneration, KU Leuven, Leuven, Belgium, 3Department of Medical Microbiology, the Netherlands Reference Laboratory for Bacterial Meningitis, CINIMA, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, 4Sanquin Research, Department of Blood Cell Research, Amsterdam, The Netherlands, and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.

Heterozygous mutations in the soluble complement regulator factor H (FH) are commonly associated with severe complement-related diseases such as atypical hemolytic uremic syndrome (aHUS) and age-related macular degeneration. We have discovered an anti-FH monoclonal antibody (mAb) that enhances FH function which could be important for clinical use. In ELISA, by measuring C3 deposition on LPS-coated surfaces, the potentiating anti-FH mAb could inhibit alternative pathway-mediated complement activation.

Importantly, the mAb neither affects FH cofactor activity in fluid phase, nor causes inhibition by consumption of circulating C3. Addition of the potentiating mAb prevents complement-mediated hemolysis of sheep erythrocytes induced by aHUS patient sera. Moreover, C3b deposition on human endothelial cells (HUVEC) incubated with patient sera is prevented by enhancing FH function with the mAb. In both ELISA and surface plasmon resonance (SPR), binding of the mAb to FH increased the affinity of FH for C3b. And the degradation of the alternative pathway convertease (C3bBb) by FH is increased. Studying recombiant FH with aHUS mutations shows that the mAb also potentiates FH function of these mutated FH. In contrast to Eculizumab, a well-known complement inhibitor used in the clinic, our mAb did not affect the bactericidal activity of normal human serum against Escherichia coli or Neisseria meningitidis and might therefore be a safer alternative to treat complement mediated diseases. In conclusion, our unique potentiating anti-FH mAb might serve as an alternative therapeutic for complement-mediated diseases to inhibit unwarranted complement activation on human endothelial cells while maintaining complement-mediated clearance of bacteria.

P.C2.04.05 Understanding the importance of B lymphocytes in the development of Type 1 Diabetes
L. Egia-Mendikute1, B. Arza, E. Rosell-Mases1, M. Carral-Pujol2, C. Vived1, A. Pansà1, C. Morà1, J. Carrascal1, T. Stratmann1, D. Serreze2;
1University of Lleida & IRBLleida, Lleida, Spain, 2University of Barcelona, Barcelona, Spain, *The Jackson Laboratory, Bar Harbor, Maine, United States.

Introduction: To analyze through which mechanisms B lymphocytes participate in Type 1 Diabetes (T1D) development, we have generated two B lymphocyte transgenic mice, the 116C-NOD and the NOD-Peri. In 116C-NOD mice, which bears immunoglobulins reactive to a non yet defined B-cell autoantigen, late disease onset and a decrease of disease incidence is found in both genders compared to the wild-type NOD mice. In contrast, the NOD-Peri mouse, in which the Immunoglobulins have specificity for penicillin, an acceleration of T1D onset and an increase of disease incidence are observed in both genders.

Methods: Phenotypic flow cytometry analysis, proliferation and cytokine release assays were performed on B and T lymphocytes from mechanically disrupted spleens and pancreatic islets of prediabetic NOD, 116C-NOD and NOD-Peri mice.

Results: Significant functional and phenotypic differences were observed between both transgenic mouse models. Islet-infiltrating B-lymphocytes (iBLs) from 116C-NOD displayed lower expression of FAS, CD86, H-2Kk and H-2Ld compared to NOD mouse, whereas increased expression of the same molecules was detected in iBLs from NOD-Peri. Moreover, the number of iBLs expressing BAFF, BFF, and TAC molecular markers was also higher in 116C-NOD compared to NOD and NOD-Peri mice. Moreover, proliferation assays showed high number of NOD-Peri mice B cells to produce large amounts of cytokines and to induce T cell activation, compared to the other models.

Conclusions: In NOD-Peri mice, B-lymphocytes have an activated phenotype and support accelerated T1D development. In 116C-NOD mice, B-lymphocytes display an anergic like phenotype, delaying T1D onset and decreasing disease incidence.
Conclusion and XIAP, demonstrating blocked apoptosis in GPA PMN. In contrast, necroptosis executers RIP3 and MLKL showed an increased expression in GPA PMN.

\[\text{cIAP1/2; XIAP; MLKL}\]

polyangiitis (GPA) are necrotizing granulomatous inflammation and necrotizing autoimmune vasculitis. Here we provide evidence, that necroptosis,

\[\text{pro-}
\]

Next to these genetic variants, anti-factor H (FH) auto-antibodies are detected in 10% to 50% of these patients. In aHUS these auto-antibodies are associated with extra-renal

\[\text{is not associated with a deletion of the}\]

Our results showed that anti-AQP4 seroreactivity was 3.9%. The 5 seropositive patients were women and had a mean age of 40 years (26-56). Three patients had an optic-sensory impairment and two had an isolated myelitis. It was the first clinical episod for 3 patients/S. In one case of optic-sensory impairment, seroreactivity was detected in a second sample performed one year after an initial seronegativity. The diagnosis of NMOSD was made for the 5 cases.

In conclusion, it seems that anti-AQP4 has a low seroreactivity in our country. However, this marker appears to be clinically relevant for NMOSD diagnosis, especially in case of incomplete and atypical presentation. We also demonstrate the interest of repeating anti-AQP4 screening after an initial seronegativity in front of highly suggestive clinicoradiological features.

Background: Endothelial cells (EC) are important contributors to inflammation via expression of inflammatory mediators, including chemokines and adhesion molecules. Production of inflammatory mediators can be induced via LTβR ligation, resulting in activation of canonical NF-κB signaling. However, the relative contribution of the individual NF-κB pathways to inflammatory activation of EC is largely elusive.

Objective: To identify the molecular pathways by which LTβR-ligation drives inflammatory activation of EC.

Methods: EC were stimulated with LTβ or LIGHT to activate LTβR, followed by analysis of downstream NF-κB signaling pathways and expression of inflammatory mediators. To repress canonical NF-κB signaling an IKKβ-inhibitor was used, and noncanonical NF-κB signaling was repressed using siRNAs targeting NFκB2. The role of NIK in LTβR signaling was investigated using inhibitors and siRNAs targeting NIK and overexpression of NIK.

Results: LTβR-ligation resulted in activation of canonical and noncanonical NF-κB signaling, and subsequent expression of inflammatory mediators. IKKβ inhibition repressed LTβR-induced inflammatory activation of EC, indicating that this process was mediated through canonical NF-κB signaling. Interestingly, NIK targeting also decreased LTβR-induced expression of inflammatory mediators, while targeting NFκB2 had no effect. Further analyses, including silencing and overexpression of NIK, demonstrated a clear role for NIK in activation of canonical NF-κB signaling by amplifying IKK complex activity.

Conclusions: These findings suggest that NIK can serve as an amplifier of canonical NF-κB signaling and associated inflammatory responses in EC, which may play an important role in the inflammatory process. Consequently, NIK may be an attractive therapeutic target.

NIK-IKK complex controls NF-κB-dependent inflammatory activation of the endothelium in response to LTβR ligation

P. Kucharzewska1, C. K. Morlace2, C. M. M. Jeukend3, J. van Hamburg4, S. W. Yst5, H. Olsson6,7,8,9

1Astrazeneca R&D, Gothenburg, Sweden, 2AMC Amsterdam, Amsterdam, Netherlands.

The results of this study show that NIK can serve as a potential therapeutic target for inflammatory diseases, as it is involved in the regulation of inflammatory responses.

1NIK-IKK complex controls NF-κB-dependent inflammatory activation of the endothelium in response to LTβR ligation

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6NIK-IKK complex controls NF-κB-dependent inflammatory activation of the endothelium in response to LTβR ligation

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8NIK-IKK complex controls NF-κB-dependent inflammatory activation of the endothelium in response to LTβR ligation

9NIK-IKK complex controls NF-κB-dependent inflammatory activation of the endothelium in response to LTβR ligation

**Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands**
POSTER PRESENTATIONS

P.C.2.04.11
CXL4 in Tunisian patients with systemic sclerosis
L. Loudhar1, J. Nammouchi1, I. Ben Ghorbel1, I. Ayadi1, H. Lhamar1, M. H. Houmar1, M. Kelle-Sellami2
1Immunology Department, La Rabta hospital, Tunis, Tunisia, 2Internal Medicine department, La Rabta hospital, Tunis, Tunisia.

Introduction: We aimed, first to assess the CXL4 level in Tunisian patients with systemic sclerosis (SSc) comparing to healthy controls and to patients presenting other clinical conditions; and second to search correlations between the level of CXL4 and the clinical manifestations of the disease. Methods: We enrolled 50 patients with SSc and with no other connective tissue diseases associated. All patients met the 2013 ACR/EULAR classification criteria. We also recruited 30 age- and sex-matched healthy controls, 36 patients with rheumatoid arthritis (RA), and 27 patients with Sjögren’s syndrome (SS). Levels of CXL4 were determined using ELISA (R&D Systems)™. Results: Patients were 47 women and 3 men with an age range of 50.1 years. The mean level of CXL4 in patients with SSc was 47.80 ± 18.27ng / ml. It was significantly higher than that in healthy controls (38.34 ± 15.83ng / ml) in patients with SLE (25.46 ± 16.09 ng / ml) and patients with RA (39.28 ± 11.91ng / ml) (p = 0.021, <0.001 and =0.026 respectively). There was no significant difference between the mean level of CXL4 in patients with SSc and in patients with SS. There was no statistically significant correlation between CXL4 levels and the various clinical manifestations of the disease. Conclusion: CXL4 appears as a potential biomarker for SSc, however it is not correlated to the clinical phenotype of the disease in our cohort.

P.C.2.04.12
Bruton’s Tyrosine Kinase (BTK) regulates the NLRP3 inflammasome directly through NLRP3 tyrosine phosphorylation
X. Lia

Department of Immunology, Tübingen, Germany.

The NLRP3 inflammasome is an inflammatory machinery participating in the pathogenesis of many inflammatory diseases. Its activation involves NLRP3 recruiting the adaptor ASC, caspase-1 binding and auto-protoeolytic activation and finally IL-1β and IL-18 maturation. However, the regulatory mechanisms of this vital inflammatory process are poorly understood. In previous studies, we identified BTK as a novel regulator of the NLRP3 inflammasome. Pharmacological and genetic BTK ablation attenuated caspase-1 activation and IL-1β maturation. BTK directly interacts with NLRP3 and ASC. In the current study, we further found that tyrosine phosphorylation of NLRP3 was significantly enhanced by the presence of BTK in co-transfected HEK293T cells, and this phosphorylation was diminished by BTK inhibitors, but not the NLRP3 inhibitor MCC950. Furthermore, a time-course pattern of increased tyrosine phosphorylation of endogenous NLRP3 was observed in human and mouse primary cells in response of Nigerian. However, NLRP3 phosphorylation was reduced in immune cells from genetically deficient human XLA patients and Btk knockout mice. Using truncated and tyrosine-mutated NLRP3 constructs, we hope to identify the exact phosphorylation site of NLRP3 that BTK targets and work out how NLRP3 tyrosine phosphorylation by BTK regulates inflammasome activity. Overall, our data contribute to a better understanding of the BTK regulation mechanism of NLRP3 activation. Given the clinical availability of FDA-approved BTK inhibitors this may pave the way for new treatment strategies in NLRP3 inflammasome-linked inflammation.

P.C.2.04.13
Down-modulation of autoreactive B-cells by protein-engineered chimeric molecules in mouse model of Type 1 Diabetes
I. Manoylov1, G. Boneva2, N. Mihaylova1, I. Doytchinova2, A. Tchorbanov2
1The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria, 2Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria, 3National Institute of Immunology, Sofia, Bulgaria.

Introduction: Type 1 diabetes (T1D) is characterized by autoimmune attack against the insulin-producing beta-cells of the pancreas. One of the main beta-cells’ autoantigens in T1D is glutamic acid decarboxylase (GAD65) - a membrane-bound enzyme catalyzing the formation of gamma-aminobutyric acid. Autoreactive B lymphocytes play major role in the pathogenesis of the disease. They produce autoantibodies against several autoantigens. B cells can activate T cells and can modulate the immune response via cytokine production. Thus, eliminating autoreactive B lymphocytes may serve as a potential treatment against T1D. Downregulation of murine B cells is accomplished via the activation of the negative receptor Fc-gammaRIIB. Logically, this receptor could be a potential target for suppression of autoreactive B lymphocytes. Materials and methods: We constructed chimeric protein molecules, containing a monoclonal antibody specific for the mouse inhibitory receptor Fc-gammaRIIB, coupled to peptide epitopes derived from GAD65 protein. The ability of these molecules to modulate the immune response was tested in an induced murine model of T1D. The parameters of this mouse model were obtained by FACS analysis, ELISPOT and proliferation assays. Results: The chimeric molecules, presented in this study, bind GAD65 - specific B-lymphocytes and suppress selectively their proliferation by co-crosslinking of the inhibitory Fc-gammaRIIB and the B-cell receptor. Conclusions: The aim of our study was to construct chimeric molecules, using antibody against Fc-gammaRIIB conjugated to GAD65 epitopes. The chimeric molecules were expected to suppress specifically autoreactive B-cells in a mouse model of T1D. This treatment presents a novel specific therapy for autoimmune diabetes.

P.C.2.04.14
The macrophage migration inhibitor factor pathway in human B cells: tight control and dysregulation in multiple sclerosis
L. Rijvers1, M. Mielief1, R. van der Vuurut de Vries1, M. Stéphant1, J. van Langelaar1, A. F. Wierenga-Wolf1, J. M. Hogervoort2, A. Geurts-Moespot3, E. C. Sweep4, R. A. Hintzen2, M. M. van Luijn5
1Department of Immunology, MS Center Erasmus, Erasmus MC, Rotterdam, Netherlands, 2Department of Neurology, MS Center Erasmus, Erasmus MC, Rotterdam, Netherlands, 3Department of Chemical Endocrinology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.

In multiple sclerosis (MS), B cells survive peripheral tolerance checkpoints to mediate local inflammation, but the underlying molecular mechanisms are underexplored. In mice, the macrophage migration inhibitor factor (MIF) pathway controls B-cell development and the induction of experimental autoimmunence encephalomyelitis. How the MIF pathway is controlled in human B cells and contributes to disease onset in MS patients remains highly elusive. Here, we show that human MIF receptor CD74 was downregulated in B cells of patients with clinically isolated syndrome (CIS) patients who rapidly developed MS (n=16) as well as clinically definite MS patients (n=15). Transitional and naive mature B cells displayed the highest CXCR4/CD74 expression ratios compared to class-switched and non-class-switched memory subsets in these patients, implying that this CXCR4 activity. Overall, our data contribute to a better understanding of the BTK regulation mechanism of NLRP3 activation. Given the clinical availability of FDA-approved BTK inhibitors this may pave the way for new treatment strategies in NLRP3 inflammasome-linked inflammation.

P.C.2.04.15
Evaluation of MAP3K in the context of CD25 expression on natural Tregs for the immune tolerance development in patients with recurrent pregnancy loss
R. Sasurkova1, A. Velichkov1, M. Mutharova1, M. Guenova1, I. Antonova2, G. Nikolov1, A. Mihova1, V. Terzieva2
1Institute of Biology and Immunology of Reproduction “Acad. Kiril Bratanov”, Sofiya, Bulgaria, 2National Specialised Hospital for Active Treatment of Haematological Diseases, Sofia, Bulgaria, 3Center for Reproductive Biology and Medicine “Reprogrammed”, University Hospital Lozenetz, Sofia, Bulgaria.

Introduction: The establishment of an immune tolerant milieu is a conditio sine qua non for the development of pregnancy. Among multiple factors involved, the population of regulatory T cells (Tregs) is of critical importance. A particular subset of Tregs, natural Tregs (nTregs), is shown to be impaired in patients with recurrent pregnancy loss (RPL). The present study is aimed at evaluating MAP3K signaling in this process. Materials and Methods: PBMCs from 10 patients with RPL (24-37yrs) and 10 age matched controls (HC) with history of successful pregnancy were stained with anti-CD3/CD4/CD45RA/CD25/Foxp3/MAP3K antibodies. Flow cytometry analysis was done by FlowJoV10 and confocal microscopy. FACS and statistical analyses were done by FlowJoV10 and GraphPad®7 software. Results: The proportion of nTregs in patients was found decreased in comparison to HC (p<0.05). In controls, but not in patients, the majority of cells were CD25+. Conclusion: The evaluation of MAP3K in study groups showed differences between CD25+ and CD25- subsets expressed mainly in anti-CD3/CD25 stimulated cells that were further evidenced by the confocal microscopy. Conclusions: Our results indicate variations in MAP3K expression that might be associated with the expression of CD25 on nTregs. Further experiments are envisaged to precisely analyze their impact on nTregs for the immune tolerance establishment. Acknowlediments:“Program for Support of Young Researchers and PhD Students at the Bulgarian Academy of Sciences (Grant no. 17-118/2017)”.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 341
The obtained results indicated the features of induced early stage of apoptosis of T-cells by transferring of autologous apoptotic cultures in vitro in norm and in rheumatoid and dexamethasone-stimulated cultures obviously increased the levels of early apoptosis of T-cells in secondary-induced CFSE+ culture. In RA, it was demonstrated a marked level of early apoptosis of T-lymphocytes in primary-induced donor-culture, stimulated with anti-CD3 antibodies (CFSE-), and secondary-dexamethasone had a modulating effect. Determined that under the equal conditions, transfer of stimulated by anti-CD3 antibodies cells significantly increased the sensitivity of T-cells to apoptosis, whereas stimulation by anti-CD3 antibodies in norm, it was established the possibility to induce early apoptosis of T-cells by transferring the cellular and humoral components of the autologous apoptotic cultures. It was established for tumor-cell-targeting. To circumvent severe side effects that occurred in earlier trials, a novel platform was developed where T-cells are equipped with a universal chimeric antigen receptor (UnICAR). Instead of binding directly to a certain structure on the target cell surface, UnICAR T-cells can be switched on and off by administering a targeting module (TM) consisting of a peptide epitope recognized by the UnICAR T-cell and a binding moiety directed against a distinct structure on the target cell surface. We determined the extracellular domain of the myelin oligodendrocyte glycoprotein (MOG) antigen as TM for specific depletion of B-cells carrying anti-MOG antibodies. We transduced a human B-cell line with an artificial anti-MOG T(H)2-homing TM, and we could demonstrate the depletion of MOG antibody body for in vivo studies we achieve a constant efficient reduction of UnICAR T-cells against self-MOG positive B-cells in a strictly target-dependent and target-specific manner. Binding capability of the TM was assessed by flow cytometry. We demonstrated that the UnICAR system is not restricted to cancer therapies and that antigen-specific depletion of auto-reactive B-cells is possible and considered to be used in autoimmune diseases, too.

The culture dish surface influences the phenotype and cytokine production of immunogenic and tolerogenic monocyte-derived dendritic cells

Introduction. Type 1 diabetes mellitus (T1DM) is a T cell-mediated autoimmune disease in which the immune system attacks the insulin producing β cells. The role of cytokines in T1DM pathogenesis is still insufficiently known. In this study, we aimed to analyze cytokine profile in patients with T1DM and to compare it with those in healthy subjects. Materials and Methods. The study group included 12 patients with DM type 1 and 20 healthy controls. Cytokine levels were determined in supernatants obtained from stimulated PBC of T1DM patients produce more proinflammatory cytokines IL-6, IL-10, TNF-α and INF-γ than in controls. The mean production of IL-10 and TNF-α in PHA-stimulated blood cells of T1DM patients tended to be higher than in controls, but statistically significant difference was obtained only for IL-6 (4295±2744 vs 1367±1133 pg/mL, p=0.040). Conclusions: The unstimulated peripheral blood cells of T1DM patients produce more pro-inflammatory cytokines IL-6, IL-17A, and TNF-α. In vitro stimulated cytokine pattern of T1DM patients is different from healthy controls. The beneficial effect seemingly is not restricted to cancer therapies and that antigen-specific depletion of auto-reactive B-cells is possible and considered to be used in autoimmune diseases, too.

Influence of autologous apoptotic cells on the parameters of early apoptosis of T-lymphocytes from healthy individuals and patients with rheumatoid arthritis

The subject of the study were blood samples from patients with RA and healthy women, matched by age. Primary-induced apoptotic cultures (CSF-) cultivated during 3 days, then their cellular and humoral components separately transferred to recipient cultures. We evaluated with CSF-., and secondary-induced culture (CSF+), compared both to the initial level of apoptosis and under transferring of unstimulated cells. Transfer of supernatants from autologous apoptotic anti-CD3- and dexamethasone-stimulated cultures obviously increased the levels of early apoptosis of T-cells in secondary-induced CSF+ culture. The obtained results indicated the features of induced early stage of apoptosis of T-cells by transferring of autologous apoptotic cultures in vitro in norm and in rheumatoid and dexamethasone-stimulated cultures obviously increased the levels of early apoptosis of T-cells in secondary-induced CSF+ culture. It is accumulated data about the lesion in apoptosis of peripheral blood mononuclear cells in patients with rheumatoid arthritis (RA). On this basis, we investigated the response of native T-cells (recipient-culture) in vitro under the transference of the autologous apoptotic cultures (unstimulated, anti-CD3- (1mg/mL) and dexamethasone- (1±10-4 M) stimulated donor cultures under conditions of crowding and depleted medium).

The subject of the study were blood samples from patients with RA and healthy women, matched by age. Primary-induced apoptotic cultures (CSF-) cultivated during 3 days, then their cellular and humoral components separately transferred to recipient cultures. We evaluated with CSF-., and secondary-induced culture (CSF+), compared both to the initial level of apoptosis and under transferring of unstimulated cells. Transfer of supernatants from autologous apoptotic anti-CD3- and dexamethasone-stimulated cultures obviously increased the levels of early apoptosis of T-cells in secondary-induced CSF+ culture. The obtained results indicated the features of induced early stage of apoptosis of T-cells by transferring of autologous apoptotic cultures in vitro in norm and in rheumatoid and dexamethasone-stimulated cultures obviously increased the levels of early apoptosis of T-cells in secondary-induced CSF+ culture.
Interleukin-30 suppresses T cell activation in murine primary biliary cholangitis

H. Chen, Y. Chang
Department of Clinical Laboratory and Medical Biotechnology, College of Medicine, Taipei, Taiwan.

Primary biliary cholangitis (PBC) is a chronic liver autoimmune disease. Our previous study found that PBC mice administered with adeno-associated virus-expressing IFN-γ (AAV-IFN-γ) showed a severe disease performance in early phase but subsequently leads to downregulation of chronic inflammation with an increase of interleukin-30 (IL-30). IL-30, also called IL-27p28, has been shown to attenuate liver injury and fibrosis. In this study, we investigated whether IL-30 had an immunosuppressive function in PBC by administering mice expressing AAV (AAV-mIL-30) to 2-OA-OVA immunized PBC mice. At first, we defined the immunosuppressive function of AAV-mIL-30 in vivo by a well-known conA-induced hepatitis mouse model. The results showed that serum levels of IFN-γ and IL-12 were decreased in AAV-mIL-30 receiving conA-induced hepatitis mice. In addition, the activation marker (CD25) of NK cells and CD4+ T cells in liver of AAV-mIL-30 receiving mice were also decreased. In PBC, the expression of CD25 and IFN-γ secretion in CD4+ T cells were decreased in mice administered with AAV-mIL-30 three weeks post 2-OA-OVA immunization. Moreover, the frequency of CD4+CD25+ regulatory T cells was increased. These results suggested that IL-30 could suppress the activation and IFN-γ production of T cells and induce Tregs. Hence, IL-30 could be a novel therapeutic cytokine in PBC.

PTPN22 negatively regulates immune complex-induced T cell proliferation by modulating dendritic cell antigen presentation and conjugate formation

F. Clarke, H. Purvis, C. Sanchez-Blanco, E. Gutierrez Martinez, G. Cornish, P. Guermonprez, A. Cope
King's College London, London, United Kingdom.

The C185T single nucleotide polymorphism in the hematopoietic tyrosine phosphatase PTPN22 confers an enhanced susceptibility to multiple autoimmune diseases including rheumatoid arthritis and type 1 diabetes. Many of the associated autoimmune diseases have an autoantibody component to their pathology. Fc receptors (FcRs) recognize autoantibodies when bound to autoantigens on tumour cells mediating FcγRs signals via Src and Syk family kinases, leading to antigen uptake, presentation and cytokine secretion. PTPN22 negatively regulates Src and Syk family kinases proximal to immunoreceptor signalling cascades. We therefore hypothesised that PTPN22 regulates immune complex induced FcR responses in dendritic cells (DCs). Bone marrow derived DCs (BMDCs) from wild type and Ptpn22−/− mice were pulsed with ovalbumin:anti-ovalbumin immune complexes (ova ICs). Co-culture with WT CD4+ OT-II T cells revealed that ova IC pulsed Ptpn22−/− BMDCs were less capable of inducing T cell proliferation compared to ova IC dependent DC-T cell conjugates. These findings highlight PTPN22 as a regulator of FcR mediated responses and provide a link between the association of PTPN22 with autoantibody associated autoimmunity diseases.

Circulating dendritic cells of patients with multiple sclerosis show inflammation-dependent gene expression changes following transmigration across an in vitro blood-brain barrier

M. De Laere1, S. Van Laere1, J. Derdelinckx2, B. Willekens3, Z. Benemann1, N. Coels1
1University of Antwerp, Wilrijk, Belgium, 2Antwerp University Hospital, Edegem, Belgium.

Control of lymphocyte entry and migration into the brain is vital to regulate protective and pathologic responses. Of interest is the finding that increased numbers of dendritic cells (DC) are present in the brain during neuroinflammation. Moreover, this has been associated with the regulation of local disease processes. But what prompts cells to enter the brain? In current study, we aim to delineate genetic mechanisms involved in transmigrating and non-migrating DC using an in vitro model for the blood-brain barrier (BBB) and transcriptome profiling. Our findings demonstrate that circulating DC of patients with relapsing-remitting multiple sclerosis (RR-MS) and chronic progressive MS (CP-MS) show an increased expression of chemokine receptors CCR2 and CCR7. This finding is paralleled by augmented chemotaxis towards the respective chemokine ligands by DC from MS patients as compared to DC from healthy controls (HC). By means of an in vitro BBB model, 2 different subtypes of DC are isolated, namely migrating and non-migrating DCs, by using chemokinetic effect on GABAergic neurons. The capacity of PTPN22 to suppress gene expression changes following transmigration is evidenced.

Mechanism of GP30 interaction and regulation of its downstream signalling

F. Dekhoda1, N. Durisic1, Y. Chhabra2, A. Brooks1
1The University of Queensland, Diamantina Institute, wooloongabba, Australia, 2The University of Queensland, Queensland Brain Institute, St Lucia, Australia.

GP30 is an archetypal member of the tall cytokine receptors and serves as a signal transducing subunit for IL-6, IL-11, IL-27, IFN-γ, and others. Physiologically, cytokines signalling via GP30 are involved in autoimmune and inflammatory diseases, including rheumatoid arthritis, inflammatory bowel diseases, and allergic asthma. Although numerous structural studies have elucidated dynamics of GP30 receptor complex, little is known about its activation and regulation of signal transduction. For this purpose, super-resolution microscopy aided single particle tracking (PALM) of GP30 molecules was performed on non-stimulated and stimulated HEK293 cells to analyse diffusion properties of the receptors on the cell membrane. To analyse the signal transducing orientation of GP30, the extracellular domain of receptor was swapped with the leucine zipper dimerisation domain of c-Jun transcription factor to generate GP30 dimers on the cell surface of pro-B cell line, Ba/F3.
The effect of length, charge, and rotation of the GP130 extracellular juxtaplambrane region was investigated resulting in identification of active and inactive receptor configurations. Variable rotation of the GP130 transmembrane and intracellular domains induced by alanine insertions mimicking cytokine induced activation led to differential activation of Jak/STAT and MAPK signalling pathways and generated distinct proliferative responses in Ba/F3 cells. These models with FRET reporters fused after the JAK binding Box2-1 region were generated and movements of the intracellular domains of GP130 with associated JAK kinases were assessed. This is the first study aimed at determining the precise movements in GP130 receptor and will allow for a targeted drug design in near future.

P.C.02.05.08
Up-regulation of EP2 and EP3 receptors in human tolerogenic dendritic cells boost the immunosuppressive activity of PGE2
G. Flórez-Grau1*, R. Cabezas2, K. E. Borgman2, E. Espahia2, J. L. Lazo2, M. F. García-Pardo2, D. Benitez-Ribas1,
1Departamento de Farmacología y Toxicología, Facultad de Ciencias, Universidad de Salamanca, Salamanca, Spain, 2Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), Barcelona, Spain, 3Institució de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Introduction: Type 1 diabetes (T1D) is an autoimmune disease which mainly affects children. Disease develops under the influence of autoantibody lymphocytes T that destroy insulin-producing β-cells. Transfer of autologous regulatory T cells (Tregs) delays the progression of the T1D. We decided to check the influence of interleukin-2 on Tregs therapy in clinical settings.

Materials and Methods: Regulatory T cells were isolated from the patients' peripheral blood with a GMP-compliant FACS-sorter. The expansion was performed under GMP conditions. Tregs were cultured for 8 days after the release of products to the clinic. Tregs and Teffs were cultured separately or co-cultured in a 1:1 ratio in the different concentrations of IL2. Survival of the cells was measured using -a-mannosidase D staining. The levels of IL2 were also measured in sera of T1D patients treated with Tregs. Results: We observed a rapid decrease in Treg cell survival without supplementation of IL2 in vitro. However, even low concentrations of IL2 were enough to limit cells death. Conclusion: Tregs were found in low levels in the sera of T1D patients. In addition, simple co-culture of Tregs with Teffs without exogenous IL2 significantly improved the viability as Teffs consist a good source of endogenous IL2. Conclusions: IL2 should be present in the culture constantly to keep Tregs viable. The levels of IL2 in vivo in T1D patients seem to be sufficient to protect Tregs transferred adaptively from cell death. This work has been supported by National Centre for Research and Development, Poland: LIDER/1601/6-14/NCRB/2015 and STRATEGMED1/233368/L/NCRB/2014.

P.C.02.05.10
Exopolysaccharides produced by Cyanobacterium aponinum from the Blue Lagoon inhibit SYK and CLEC7a expression by dendritic cells and keratinocytes and dampen the function of T-cells
A. B. Gudmundsdottir1*, A. Brynjolfsdottir2, E. O. Gilsdottir1, I. Hardardottir2, J. Freydrisdottir2,
1Faculty of Pharmaceutical Sciences, University of Iceland, Reykjavik, Iceland, 2University of Iceland, Reykjavik, Iceland.

Introduction: Regular bathing in the Blue Lagoon has beneficial effects on psoriasis. We have shown that exopolysaccharides (EPS-Ca) from one of the lagoon’s dominating microbes, Cyanobacterium aponinum, increased dendritic cell (DC) secretion of IL-10 and DC induction of Tregs at the cost of the inflammatory Th17 cells. The objective of this study was to determine how EPS-Ca affects DCs and to determine the effects of its stimulated T-cells and keratinocytes. Materials and methods: Human monocyte-derived DCs, CD4+ T-cells and adult primary keratinocytes were stimulated with IL-1β, TNF-α and LPS; antibodies against CD3 and CD28; or TNF-α and either IFN-γ or IL-17, respectively, in the presence or absence of EPS-Ca. Cytokine secretion was measured by ELISA, expression of mRNA by rt-PCR and surface expression of molecules by flow cytometry. Results: EPS-Ca decreased secretion of TNF-α, IL-1β and IL-12 (linked to DCs stimulated without EPS-Ca), and CD41+ DCs secreted more IL-10 than CD141+ DCs. EPS-Ca treatment decreased DC secretion of Dectin-1 and transcription of genes in the SYK-signaling pathway (CLEC7a and SYK). T-cells stimulated in the presence of EPS-Ca secreted less IL-10, IL-13 and IL-17 and expressed less CD69 than T-cells stimulated without EPS-Ca. EPS-Ca decreased keratinocyte secretion of CCL20 and CXCL10 and their transcription of S100A9, and autocrine CAMP (LL37). Conclusions: These results demonstrate inhibitory effects of EPS-Ca on important signaling pathways in DCs, T-cells and keratinocytes involved in the pathogenesis of psoriasis, demonstrating the potential of EPS-Ca as a drug lead for the treatment of psoriasis.

P.C.02.05.11
Interferon signature in patients with STAT1 gain-of-function mutation is epigenetically driven
E. Kaleviste1, M. Saare1, R. Leahy2, W. Ip3, G. E. Davies3, P. Peterson1, K. Kisand1,
1University of Tartu, Tartu, Estonia, 2Our Lady’s Children’s Hospital, Dublin, Ireland, 3UCL Great Ormond Street Institute of Child Health, London, United Kingdom.

STAT1 is a transcription factor that mediates signals from IFNs. While STAT1 loss-of-function mutations confer susceptibility for viral and mycobacterial diseases the gain-of-function (GOF) variants of STAT1 lead to defective Th17 cell development and chronic mucocutaneous candidiasis (CMC). In addition, these patients develop autoimmune disorders: thyroid disease, cytopenias, type 1 diabetes, SLE, vitiligo, alopecia. STAT1 GOF mutation results in hyperphosphorylation and delayed dephosphorylation of STAT1.

Gain-of-function (GOF) variants of STAT1 lead to defective Th17 cell development and chronic mucocutaneous candidiasis (CMC). In addition, these patients develop autoimmune disorders: thyroid disease, cytopenias, type 1 diabetes, SLE, vitiligo, alopecia. STAT1 GOF mutation results in hyperphosphorylation and delayed dephosphorylation of STAT1.

Conclusion: Interferon signature in patients with STAT1 gain-of-function mutation is epigenetically driven

P.C.02.05.12
The treatment outcomes in IgG4-related disease
F. Karim1, K. Banse2, S. Rombach2, M. van Herwaarden3, J. van Laar4,
1Erasmus MC Rotterdam, Netherlands and Groningen, 2Hart-Ziekenhuis Gouda, Netherlands, Rotterdam, Netherlands.

Introduction: IgG4-related disease (IgG4-RD) is an emerging systemic inflammatory disease involving nearly all organs eventually leading to fibrosis. Prompt and adequate treatment to prevent irreversible damage is therefore pivotal. To evaluate the treatment outcomes, we studied a well-defined cohort of patients with IgG4-RD. Method: 32 patients with histologically confirmed IgG4-RD diagnosed between 1999 and 2017 were included and reviewed for demographic and clinical characteristics. The response to treatment with glucocorticoids, disease-modifying antirheumatic drugs, rituximab and other therapeutic interventions were evaluated. Results: Glucocorticoids as well as rituximab appeared to be the most effective drugs for the initial treatment leading (clinical remission: complete or partial remission) in all patients. Recurrence of IgG4-RD after discontinuation of glucocorticoids was associated with worse clinical remission rates. Conclusions: Glucocorticoids and rituximab induce substantial responses as well as primary surgical intervention and radiotherapy, while the efficacy of MMF is limited. Based on the few data available, hydroxychloroquine, infliximab and thalidomide may be promising treatment options for second or third line strategies.
POSTER PRESENTATIONS

P.C2.05.13
OCA-B in the pathogenesis of Type 1 Diabetes
H. Kim, A. Shokly, C. German, D. Tantin;
The University of Utah, Salt Lake City, United States.

Introduction: Type 1 diabetes (T1D) is an autoimmune disease caused by the destruction of insulin-producing beta cells in the pancreas. The mechanisms of T1D pathogenesis remain incompletely understood. Oct1 is a sequence-specific DNA binding transcription factor that in T cells potently regulates target gene expression with a co-factor, OCA-B. Both proteins control CD4+ T cell memory. Importantly, pathogenic CD8+ memory T cell frequency correlates with severity of insulin in the non-obese-diabetic (NOD) mouse. The strongest OCA-B reporter is found in pancreas-infiltrating islet-reactive CD4+ T cells. Moreover, GWAS studies associate SNPs at multiple Oct1/OCA-B binding sites with T1D.

Materials and Methods: To study OCA-B effects on CD4+ T cell memory, WT or OCA-B KO naive CD8+ T cells were cultured with a CD3/28 for 4 days. Subsequently, the cells were rested for 8 days without stimuli, and were re-stimulated for a recall response. To investigate impacts of OCA-B in the pathogenesis of T1D, NOD mice were injected with newly developed OCA-B inhibitor peptides or controls.

Results: OCA-B loss in CD4+ T cells failed to induce recall responses in vitro. Strikingly, the inhibitor peptides reversed the elevated blood glucose in NOD mice. T cell infiltration and cytokine production in the pancreas was reduced compared to NOD mice treated with control peptide.

Conclusions: OCA-B regulates both CD4+ and CD8+ T cell memory and plays a role in T1D pathogenesis. To elucidate the mechanisms more deeply, OCA-B germline KO and T cell-specific OCA-B deleted NOD mice have been generated.

P.C2.05.14
Ethyl pyruvate treatment enhances regulatory T cell proliferation and function in type 1 diabetes
I. Koprivica, M. Vujčić, T. Šakić, D. Gajić, I. Stojanović;
institute for Biological Research "Sindija Stanković", Belgrade, Serbia.

Type 1 diabetes (T1D) is an autoimmune disease in which a strong inflammatory response causes insulin-producing pancreatic β-cell death. Ethyl pyruvate (EP), a stable pyruvate derivate, has exerted antioxidant and anti-inflammatory properties in several disease models. To test its therapeutic potential in T1D, EP was administered intra-peritoneally to C57BL/6 mice with multiple low-dose streptozotocin (STZ)-induced T1D. EP treatment decreased T1D incidence and reduced the infiltration of cells into the pancreatic islets. In vivo analysis by flow cytometry showed that the EP treatment didn’t change the number of immune cells in the spleen, pancreatic lymph nodes (PLN) or pancreatic mononuclear infiltrates (PMNI), nor the relative percentages of Th1, Th17 and Th2 cells. However, EP treatment increased the levels of regulatory T cells (Treg) in PLN and PMNI. After the EP treatment, all PLN Treg were GITR+CD127+ and an increase was noted in the percentage of CD101+Treg, indicating a stronger suppressive activity. That was confirmed by an in vitro suppression assay, in which Treg from EP treated mice showed a higher capacity to suppress effector T cell proliferation. The ratio of CD86+CD103+ and the presence of CD11b+CD62L per cell increased, which might imply an increase in Treg migration into the pancreas. However, a rise in the presence of KI67+Treg suggested that EP treatment also promotes Treg proliferation. These results show that EP treatment reduces T1D incidence in C57BL6 mice by enhancing Treg proliferation, suppressive capacity, and recruitment into the pancreas. This research is supported by MESTD, Republic of Serbia (#173013).

P.C2.05.15
The role of IL-23/Th17 pathway in Inflammatory bowel disease
Y. Lakhoua Gorgi1, M. Joudboui1, J. Abdelatif1, M. Jellouli1, T. Dhaoudi1, L. Mouelhi1, T. Ben Abdallah1, I. Sfar1;
1Research Laboratory in Immunology of Renal Transplantation and Immunopathology (LR03SP01), Tunis, Tunisia, 2Department of Gastroenterology Charles Nicolle hospital, Tunis, Tunisia

AIMS: Investigate a possible association between the functional polymorphisms: IL-17R rs70567, IL-17F rs2397084 and IL-23R rs11290926 and the susceptibility to IBD and define the impact of these genetic variants on the IBD clinical forms: METHODS: A cross-sectional case-control study involving 178 patients with IBD (108 CD and 70 UC) and 100 healthy control subjects was made. The molecular analysis was performed by PCR-RFLP. An ELISA tests were used to process to the quantitative determination of serum IL-17F and IL-23 cytokines.

RESULTS: Quantitatively, the mean level of IL-17F was significantly higher in patients than in controls (p = 0.003) and in CD patients with strictureing complications compared to other clinical forms (p = 0.006). The serum IL-23 levels were similar in patients and controls. The genotype G/G of IL-17R polymorphism and the A/G genotype of IL-17F-SNP’s seem to be associated to a higher occurrence of IBD and to the positivity of the ANCA antibodies in patients with UCs (p = 0.049 and p = 0.008). For the R381Q IL-23R polymorphism, the frequency of the A allele was significantly higher in controls than in IBD patients (p = 0.0001) suggesting a protective role of this SNP. CONCLUSION: The results of our study support the key role of IL-23Th17 pathway in the pathophysiological mechanisms of IBD. The prognostic interest of these markers deserved to be confirmed by further prospective studies on a larger cohort and by a functional analysis of IL-17F and IL-23mRNA expression.

P.C2.05.16
Sulforaphane inhibits inflammatory responses of primary human T-cells by increasing ROS and depleting glutathione
J. Liang1, B. Ibrahim2, E. Balt1, J. Ziegler1, K. Hübner1, N. Blank1, B. Niesler1, G. Wabnitz1, Y. Samstag2;
1Institute of Immunology, Heidelberg, Germany, 2Department of Rheumatology, Heidelberg, Germany, 3Department of Human Molecular Genetics, Heidelberg, Germany.

Sulforaphane (SFN), a compound in plants of the brassicaceae family, was reported to suppress cancer cell growth. Information about the relevance of SFN for human T-cells is limited. This is surprising as T-cells play a critical role in tumor control. We, therefore, investigated the effects of SFN on human untransformed peripheral blood T-cells. While SFN did not affect cytokine expression and did not interfere with early T-cell activation, it affected later activation events as upregulation of CD69 and CD25. The inhibitory effects of SFN could be rescued by thiol-containing antioxidants. In line with that finding, SFN led to an increase of intracellular reactive oxygen species (ROS) and a marked decrease of glutathione. Consistently, increased global cysteine sulfenylation was detected. Importantly, a major target for SFN-mediated protein cysteine sulfenylation is eIF2α, a translation factor involved in the regulation of Th17-related genes. Moreover, costimulation-induced STAT3 phosphorylation was significantly inhibited by SFN. In an unbiased gene expression signature analysis, we indeed found that Th17-related genes were predominantly inhibited by SFN. Since IL-17 and ROS regulation may be attractive targets for treating rheumatoid arthritis (RA), we tested the effect of SFN on whole blood from RA patients and found an increase in intracellular ROS levels in T-cells. Moreover, costimulation-induced expression of IL-17 was markedly decreased in SFN-treated T-cells from RA patients. Taken together, our study shows that SFN may act as a promising substance for therapeutic immune suppression via regulating the redox balance in human T-cells.

P.C2.05.17
Novel biomarkers of disease combined with ligand-directed targeted therapy for the control of autoimmune arthritis
S. H. Venkatesha1, S. Dudics1,2, R. R. Meka1, K. D. Moudgil1;
1University of Maryland School of Medicine, Baltimore, Baltimore, United States, 2Baltimore Veterans Affairs Medical Center, Baltimore, United States

Introduction: Rheumatoid arthritis (RA) affects millions of people worldwide. A key event here is the breakdown of self-tolerance leading to anti-self-reactivity and tissue dysfunction/damage in the synovial joints. Current biomarkers for the diagnosis and prognosis of RA have inherent limitations. Similarly, the presently used drugs against RA are potent but their prolonged use is associated with severe adverse effects. Thus, there is a need for new biomarkers of disease and novel approaches to improve the therapeutic index of anti-arthritic drugs.

Materials and Methods: We addressed both these issues using the adjuvant-induced arthritis (AA) model of RA. We examined the micro-RNA (miRNA) expression profiles of the peripheral lymphoid cells of untreated/ treated arthritic and control rats, using miRNA-microarray. The data was subjected to statistical and bioinformatics analyses. Results: We identified 8 specific miRNAs that were upregulated upon arthritis development. Collectively, these miRNAs targeted T cell response, angiogenesis, and bone remodeling pathways, but individual miRNA specifically affecting Th17/Treg differentiation was also identified. Six of above 8 miRNAs were inhibited following arthritis treatment. For RA therapy, we employed a novel peptide ligand that preferentially homes to inflamed joints after systemic administration, to direct drug-entrapping liposomes into the joints. Arthritic rats treated with these liposomes showed markedly reduced severity of arthritis and decreased systemic toxicity compared to rats treated with free drug.

Conclusion: Our study has unraveled novel biomarkers of disease development and therapeutic response as well as an improved targeted therapy for autoimmune arthritis. (Acknowledgement: grants from NIH and Veterans Affairs)
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P.C.2.05.18
miR-130a dysregulation contributes to CD1c+ dendritic cell activation in Sjögren’s Syndrome

1Department of Rheumatology & Clinical Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands, 2Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands, 3Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands.

Introduction. Primary Sjögren’s syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands and mucosal dryness. As down-regulation of toll-like receptor 3 (TLR3) expressing dendritic cells (dDCs) is one of the cardinal pathological hallmarks in pSS pathogenesis, we investigated miRNA expression in isolated CD1c+ dDCs from pSS patients. Methods. dDCs were isolated from peripheral blood of two independent cohorts of patients and healthy controls (HC): a discovery cohort (15 pSS, 6 HC) and a validation cohort (14 pSS, 3 HC). qPCR-based profiling of 758 miRNAs was performed in the discovery cohort. A selection of 18 differentially expressed miRNAs was measured in the validation cohort using a custom array. To study miR-130a regulation, isolated dDCs from HC were stimulated with different TLR ligands. To discover novel targets of miR-130a, HEK-293T cells were transfected with a miR-130a mimic and protein synthesis was analyzed using pulse labeled stable isotope labelling by amino acids in cell culture (pSilico). Results. 36 miRNAs were downregulated in pSS patients versus HC in the discovery cohort. Of the 18 selected miRNAs for replication, the decreased expression of miR-130a and miR-708 was validated. dDC activation through TLR3 and TLR7/8 downregulated miR-130a. Transfecting HEK-293T cells with miR-130a identified downregulation of proteins involved in NF-κB signalling. Conclusions. We here show that miR-130a and miR-708 are decreased in pSS dDCs, which is induced by TLR-activated cell injury. In addition, we provide evidence showing that miR-130a targets mediator of inflammatory response in NF-κB signalling and dDC activation at the protein level.

P.C.2.05.19
Type 1 diabetes and related autoantibodies by the age of 13 years are not associated with probiotics administration in infancy

E. Savilahti1, M. Knip1, E. M. Savilahti1, A. K. Kukkonen1, M. Kuitunen2
1Children’s Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland, 2Skin and Allergy Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Helsinki, Finland.

Intestinal microbiota is thought to play a role in the development of type 1 diabetes (T1D), but prospective evidence on human studies is scarce. We used the data and a clinical double-blind randomized placebo-controlled trial on primary allergy prevention (n=1223) to investigate whether administration of a mix of pro- and probiotics during late pregnancy and the first 6 months of life was associated with prevalence of T1D during 13-year follow-up. Fourteen children had been diagnosed with T1D by November 2017, when the youngest participant was 13 years old. All those who developed T1D had complied to the intervention. Of them, 7 had received the active probiotic preparation. Factors known to have an effect on intestinal microbiota were similar among those developing diabetes and the remaining participants. In the 649 blood samples taken at the age 5, we analyzed GADA (96-585), IA-2A, IA, IAA, ZnT8A 6.2 antibodies. Islet cell antibodies were measured only among those with positivity in any of the afore mentioned measurements. Levels above normal were found in altogether 25 children; 7 of them having increase in two or more measurements. Among these children, 12/25 with positive autoantibodies and 3/7 with ≥ 2 positive autoantibodies had received probiotic treatment during infancy. Probiotic or placebo treatment was thus not associated with positive autoantibodies at the age of 5 years. In conclusion, we found no effect of probiotic treatment in development of T1D until the age of 13 years or on islet cell autoimmunity at age 5 years.

P.C.2.05.20
Systemic level of IL-12p40 and its genetics polymorphisms in relapsing remitting multiple sclerosis

L. D. Miteva1, A. G. Trenova1, S. A. Stalnov1
1Medical Faculty, Stara Zagora, Bulgaria, 2Medical University, Medical Faculty, Plovdiv, Bulgaria, 3Trakia University, Medical Faculty, Stara Zagora, Bulgaria.

Introduction: Cytokines are tightly involved in the pathogenesis of multiple sclerosis as an inflammatory demyelinating disease. IL-12p70 and IL-23 share an IL-12p40 subunit. Two functional polymorphisms are identified within the IL12B gene encoding IL-12p40, rs17865058 and rs3212227. We aimed to determine the systemic IL-12p40 level among patients with relapsing-remitting multiple sclerosis (RRMS). Materials and Methods: The genotyping was performed on 156 patients and 370 controls by PCR-based methods. Serum IL-12p40 concentrations were measured during remission of the disease by ELISA. Results: IL-12p40 was significantly higher in RRMS patients than in controls (188.4 ng/ml, 95% CI: 125.2-251.7 vs. 149.8 ng/ml, 95% CI: 116.2-183.2, p<0.0001). There was no statistically significant difference in serum IL-12p40 between patients without (597±15.5) and with (556±19.3) moderate/disable (EDSS>2.5) disability (p=0.821). The IL-12p40 levels decreased into the following order of genotypes of rs3212227: AA>AC>CC. Significantly lower IL-12p40 in patients carriers of CC-genotype (184.5±19.9) was compared to AA-genotype (198.9±18.5) (p=0.0014) and AC-genotype (179.3±19.4) (p=0.024) was observed.

P.C.2.06.01
Therapeutic efficacy of systemic administration of orexin A in established experimental autoimmune encephalomyelitis

L. Becquet1, C. Abad2, M. Leclercq3, C. Miel1, L. Jean4, G. Riou5, A. Couvain6, G. Boyer7, Y. Tan8
1Normandie Univ, UNIROUEN, INSERM, U1234, PANTHER, Rouen, France, 2Paris-Diderot University, INSERM U1149, ImmunoInflammation Research Center (CRI), DLUH UNITY, Paris, France, 3Normandie Univ, UNIROUEN, INSERM, U1234, Rouen University Hospital, Department of Immunology and Biotherapy, Rouen, France.

Orexins (hypocretins, Hcrt) A and B are GPCR-binding hypothalamic neuropeptides known to regulate sleep/wake states and feeding behavior. A few studies have shown that orexin A exhibits anti-inflammatory and neuroprotective properties, suggesting that it might provide therapeutic effects in inflammatory and neurodegenerative diseases like multiple sclerosis (MS). MS is a highly prevalent autoimmune disease where encephalitogenic Th1 and Th17 cells trigger an inflammatory response in the CNS destroying the myelin sheath. Here, we investigated the effects of peripheral orexin A administration to mice with experimental autoimmune encephalomyelitis (EAE), a model of MS.

EAE was induced by active immunization of C57BL/6 mice with myelin oligodendrocyte glycoprotein (MOG)35-55 peptide. Orexin A was peripherally administered and the efficacy was assessed clinically, as well as by histopathology and cytokine and chemokine miRNA expression analysis in the CNS. Peripheral responses to MOG35-55 were studied by ex vivo antigen-recall assays. Orexin A strongly ameliorated ongoing EAE, limiting the infiltration of immune cells and diminishing inflammation responses in the CNS. Moreover, orexin A treatment was neuroprotective by modulating glial cells and astrocytes. Despite its strong therapeutic effects, orexin A did not impair draining lymph node cell proliferation and Th1/Th17 cytokine production in response to MOG35-55 in vitro. These results suggest that orexins may represent new therapeutic candidates that should be further investigated for MS treatment.

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P.C.2.06.02
Targeting of tolerogenic dendritic cells towards heat-shock proteins: a novel therapeutic strategy for autoimmune diseases

M. Jansen1, R. Spiering2, P. van Kooten3, J. Ludwig1, W. van Eden4, C. Hilken5, F. Broere6
1Utrecht University, Utrecht, Netherlands, 2Newcastle University, Newcastle upon Tyne, United Kingdom.

Rheumatoid arthritis (RA) is an autoimmune disease caused by faulty regulation of the inflammatory process. A promising strategy to specifically target the pathogenic T cell response, while leaving other, protective T cell responses intact, is the use of tolerogenic dendritic cells (tDCs). Since the self-antigen for RA is unknown and HSPO70 has the potential to induce antigen specific Tregs, we investigated HSPO70 as surrogate antigen. tDCs were induced by desamethasone and 1,25-dihydroxyvitaminD3 treatment of murine bone marrow derived dendritic cells (BMDCs). Subsequently, DCs were loaded with HSPO70 peptide (829-908) and the effects of tDCs on CD4+ T cells in vitro and in vivo were investigated. tDCs infected with a proteoglycan induced arthritis mouse model. tDCs exhibited a semi-mature phenotype and changed the phenotype of CD4+ T cells to an immunodulatory state, both in vitro and in vivo, when compared to mature BMDCs. T cells stimulated by tDCs showed decreased proliferation and produced more IL-10. Interestingly, more tDCs significantly reduced arthritic symptoms, suggesting that a maturation stimulus is necessary to exert their function in vivo. These results indicate that cultured tDCs are able to modulate the CD4+ T cell response towards an anti-inflammatory state. Since the mice were infected with matched tDCs we show that these DCs reduce arthritis, we believe this mechanism might contribute to the efficacy of the tDCs. Studying the immune modulatory capacity of tDCs is essential for the further development of tDCs for RA therapy.
POSTER PRESENTATIONS

P.C2.06.03
The immunomodulatory properties of the hookworm protein Na-AIP-1

G. Buitrago, S. Ryan, J. Jones, D. Picking, P. Giacomini, A. Lousakas; Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, Australia.

Introduction: Inflammatory bowel disease (IBD) is an umbrella term for a group of immune-mediated conditions, which are characterised by idiopathic chronic inflammation of the gastrointestinal tract. Current treatment protocols for IBD are often poorly tolerated or ineffective. Multiple studies have described the potential efficacy of live parasitic helmint infection in alleviating symptoms of intestinal inflammation, in both human and animal models of disease. Consequently, there is interest in identifying potential immunomodulatory properties of immune cells recruited by helmints, which could be produced as a safer and more acceptable alternative to live worm therapy. Materials and Methods: We have identified, isolated and recombinantly expressed a novel candidate molecule from the secretions of the anthropophilic hookworm Necator americanus (Na-AIP-1). Results: Recombinant Na-AIP-1 was cloned and expressed using Pichia pastoris and purified via immobilised metal ion affinity chromatography. Intra-peritoneal treatment of mice with recombinant Na-AIP-1 ameliorated clinical, immunological and histological features of disease in two distinct murine models of colitis (TNBS and T cell transfer-induced colitis). Protection against colitis required the presence of CD11c+ dendritic cells and was associated with expansion of CD4+ Foxp3+ T cells within mucosal tissue sites. Conclusions: Thus, Na-AIP-1 may represent an excellent candidate for further development as a novel treatment of autoimmune or inflammatory diseases of the intestine.

P.C2.06.04
IFN-alpha treatment has different effects on NK cell populations in relapsing and monophasic rat experimental autoimmune uveitis

M. Diedrichs-Möhling1, X. Liu2, G. Wildner1.
1Section of Immunobiology, Dept. of Ophthalmology, University Hospital, LMU Munich, Germany; 2Present address: Ophthalmic Center of the Second Hospital, Jilin University, Changchun, China.

Interferon alpha (IFN-α) is a successful therapy for ocular Behçet’s disease, but the underlying immune mechanisms are not yet fully understood. To further elucidate the therapeutic mechanisms we investigated the effect of IFN-α on a leukocyte populations in two experimental rat models of relapsing and monophagic uveitis and uveitis. Uveitis was induced by immunization with either splenic T cells of PDSA (monospecific/chronic EAU) or IRBP-peptide R14 (relapsing EAU). Recombinant human IFN-alpha was subcutaneously injected every day or every other day at different doses (1000 or 5000 IU) starting from different time points. Uveitis was graded clinically and histologically. Peritoneal exudate cells (PEC), lymph node (LN) cells and peripheral blood lymphocytes (PBL) were investigated for surface markers and cytokine expression by FACS-analysis. Preventive treatment with IFN-alpha starting with immunization significantly decreased the primary inflammation as well as relapses of R14-induced EAU, while PDSA-induced, monophasic disease was deteriorated. Lymph node cells of PDSA-immunized, IFN-α-treated rats showed more T cells expressing IFN-γ, IL-17 or both, and less T cells producing IL-10 compared to the PBS-treated control group. IFN-α treatment decreased cell populations expressing CD161 in PDSA-immunized Lewis rats, whereas in R14-induced EAU an increase of CD161+ cells. IFN-α treatment had different effects on relapsing and monophasic rat uveitis models. The deterioration of PDSA-induced EAU by IFN-α treatment might be due to an increase in leukocyte populations expressing inflammatory cytokines, and cells expressing CD161+ might have a regulatory effect in R14-induced EAU. Supported by Alexander von Humboldt/Von Siemens -Foundation

P.C2.06.05
Immune modulating effects of low level laser therapy in oral lichen planus patients

M. N. Draganova-Filipova1, M. Z. Mutafchieva1, S. Y. Bachurska1, G. T. Tomov2, P. I. Zagorcheva2; 1Department of Medical Biology, Faculty of Medicine-Medical University-Plovdiv, Bulgaria, Plovdiv, Bulgaria; 2Department of Periodontology and Oral diseases, Faculty of Dental Medicine, Medical University-Plovdiv, Plovdiv, Bulgaria, Bulgaria; 3Department of of General and Clinical Pathology, Faculty of Medicine, Medical University-Plovdiv, Plovdiv, Bulgaria, Bulgaria; 4Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University-Plovdiv, Plovdiv, Bulgaria, Plovdiv, Bulgaria,

Introduction: Oral lichen planus (OLP) is an autoimmune inflammatory disease, result of activation of T lymphocytes against unknown antigen. Therefore, destruction of the basal epithelial cells occurs and painful wounds are formed. The low-level laser therapy (LLLT) is considered as promising and harmless opportunity for OLP patients. The aim of this study was to compare the expression of pro- and anti-apoptotic markers in patient’s biopsies and the salivary levels of IL-1, Th-2, Th-17-cytokines and SIgA before and after LLLT. Material and methods: 20 OLP patients and 20 healthy donors underwent LLLT with a diode laser (810nm), 3 times weekly for a month. The biopsies were taken before and after therapy and were analyzed immunohistochemically for the p53, p63, and bcl-2-expression. The reaction intensity was measured using a semiquantitative scale. The levels of IL-1β, IL-6, IL-17, and SIgA were measured by ELISA in unstimulated whole saliva. Results: The results from immunohistochemistry showed that p53 and p63 expression was not changed (p=0.69 and p=0.42), but bcl-2-levels were increased (p<0.02) after therapy. In OLP patients cytokine levels of IL-1β (812±28 pg/ml), IL-6 (26,9±10,3 pg/ml) and IL-17 (19,0±9,7 pg/ml) were higher compared to controls (21,2±3,7 pg/ml; 14.1±6,2 pg/ml; 92,2±23,7 µg/ml before and after LLLT. Conclusions: LLLT is useful and harmless treatment modality for OLP patients, capable to balance the immune response. Keywords: immune regulation and therapy, inflammation, autoimmunity.

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P.C2.06.06
Critical role play by galectin-1 in the T cell mediated regulation of experimental colitis


Foxp3-expressing regulatory T cells (Treg) are vital for maintaining balance among tolerance, adequate immune responses, and autoimmunity. In recent years, regulatory T cells are being developed as a cellular therapy with the potential to modulated unwanted immune responses. While their homeostasis has been extensively studied, their mechanisms of immune regulation and therapy, inflammation, autoimmunity. Acknowledgements: The investigation is sponsored by Grant HO-03/2014/MU-Plovdiv and partially by National Science Fund-Bulgaria.

P.C2.06.07
Genetic microglia depletion ameliorates neuroinflammation in experimental autoimmune encephalomyelitis

J. Han, H. Lund, K. Zhu, M. Pieber, R. A. Harris, X. Zhang; Karolinska Institutet, Stockholm, Sweden.

Microglia are the principal resident immune cells in the central nervous system (CNS) and are believed to be versatile players in both inflammatory and physiological contexts. Although the dysfunction of microglia and microglia-induced neuroinflammation are implicated in the occurrence and progression of many neurological diseases, what remains largely enigmatic is the relative importance of microglia during different disease stages. We immunized CX3CR1CreER(Rosa26) onto PDSA mice with myelin oligodendrocyte glycoprotein (MOG) to induce experimental autoimmune encephalomyelitis (EAE). Microglia depletion in DTAs mice was initiated by injecting tamoxifen (TAM) for three consecutive days, resulting in almost complete loss of microglia after another 7 days. Depletion was initiated at day 5 of post-immunization during the period of disease initiation and early progression. The efficiency of microglia depletion was confirmed by using both microglia-specific P2y12 immunohistochemistry and CD11b+CD45+Ly6G+CD11c+ flow cytometry. The severity of EAE was evaluated using a clinical scoring system. At day 10 post-sacrificed and the tissues of brain and spinal cord were assessed by flow cytometry. Our preliminary results indicate that microglia-depleted mice had less severe neuroinflammation as assessed by both the clinical scores and the data from flow cytometric analyses. Our results demonstrate that microglia are involved in the initiation and early progression of MOG-EAE. The tamoxifen-inducible CX3CR1CreER(Rosa26) mice can be viewed as effective microglia-specific genetic tool to study the role of microglia in neurological diseases in vivo.
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P.C2.06.08
Adapted venom-derived mesenchymal stem cells alter the Th17/Tregs axis in patients with rheumatoid arthritis yet not in patients with systemic sclerosis - preliminary data

Medical University of Sofia, Sofia, Bulgaria.

Mesenchymal stem cells (MSCs) are fibroblast-like progenitor cells that possess the capacity to self-renew and can differentiate into several mesenchymal lineages. MSCs suppress the antigen-specific T-cell proliferation and cytotoxicity as well as inducing the generation of T reg cells. Regarding the pivotal role of the Th17/Treg axis in the pathogenesis of systemic sclerosis (SSc) and rheumatoid arthritis (RA), we aimed to study the immunosuppressive effects of adipose tissue-derived MSCs (AT-MSCs) conditioned medium on the percentage of Th(17) and Tregs obtained from patients with SSc and RA. We enrolled 5 patients who fulfilled the 2013 ACR/EULAR criteria for the classification of SSc and 13 patients matching the ACR/EULAR 2010 criteria for RA. AT-MSCs were isolated and cultured according to well established protocols. We used ELISA to quantify the cytokines produced by AT-MSCs (IL-1, IL-10, IL-4, IFN-y, IL-6, IL-8, CCL-5, RANTES, IL-17, TGF-β). Peripheral blood mononuclear cells (PBMCs) isolated from SSc and RA patients were cultured in AT-MSCs conditioned media as well as in control media. We performed flow cytometric analysis of the percentage of Th(17) and 1 Tregs and TGF-β levels produced by PBMCs were detected by ELISA. Our results demonstrated decreased percentage of Th(17) cells (p=0.012) under the influence of AT-MSCs medium in RA yet not in SSc patients. The percentage of Tregs was raised only in RA patients: CD4 FoxP3+ lymphocytes (p=0.031), CD4 FoxP3 CD25+ (p=0.001). Further, in RA patients we found considerable increase in TGF-β levels (p=0.031) produced by PBMCs cultured with AT-MSCs medium as opposed to control PBMCs.

P.C2.06.09
PD1+CXCR5 peripheral helper T-cells producing IL21 and IFNγ are increased in systemic lupus erythematosus

J. Land, M. Parvar, M. R. Ehrenstein;
Centre for Rheumatology, Division of Medicine, University College London, London, United Kingdom.

Introduction: Follicular helper T (Tfh) cells are a specialised CXCR5+ T-cell subset that provides signals to B-cells, which are known to be increased in frequency in systemic lupus erythematosus (SLE) patients. Recently, a distinct circulating subset termed peripheral helper T (Tph) cells was described which are CXCR5- but have similar functional properties to Tfh.

Methods: Flow cytometry was performed on PBMC from 43 SLE and 12 healthy controls (HC) to determine the Tfh/Tph phenotype, including expression of PD1 and CXCR5. Production of IL21 and IFNγ was determined in HC SLE and RA.

Results: The percentage of circulating CXCR5+PD1+ Tfh cells was increased 1.5-fold in SLE compared to HC. The increase in CXCR5+PD1+ Tph cell frequency was more pronounced being 4-fold higher in SLE (median 1.8%,range 0.3-9.6%) than in HC (0.4%;0.1-1.2%). SLE patients had an increased proportion of IL-21 producing Tfh and Tph cells. T-cells with high expression of PD1 demonstrated the largest proportion of IL21+ and IFNγ+ cells, regardless of CXCR5 expression. Double-positive IL21+IFNγ+ Tfh and Tph cells were also increased in SLE. The percentage of Tfh cells correlated with B-cell frequency in SLE ( spearman's r=0.5;p=0.001) but not in HC, while Tph cell frequency negatively correlated with C3 levels (r=-0.361;p=0.031).

Conclusion: Tfh as well as Tph cells are increased in SLE. The increase in IL-21+ T-cells within these two populations contained a large proportion of IFNγ producing cells. Th frequency did not correlate with disease activity, while Tph frequency correlated with C3 levels which are reduced in active disease.

P.C2.06.10
The role of the Tec kinase ITK and the transcription factor NFATc1 in disease pathogenesis of inflammatory bowel disease

K. Lechner, S. Mott, M. F. Neurath, B. Weigmann;
Department of I., Medical Clinic, Erlangen, Germany.

Introduction: ITK, a member of the Tec family kinases, is expressed in T cells and involved in Th2-type mediated immune responses. Colitis patients can be successfully treated with CsA but CD patients not. Therefore, we started to investigate the role of ITK (interleukin-2-inducible T cell kinase) and the linked transcription factor NFATc1 in experimental colitis model.

Methods: Oxazolone colitis was induced in ITK deficient mice, conditional NFATc1-ΔCD4-KO mice and controls. Disease activity was measured by means of body weight, histological and endoscopic score of inflammation activity. Lamina propria mononuclear cells (LPMC) were isolated from these mice. The rate of apoptosis induction after treatment with CsA was assessed by flow cytometric analysis of AnnexinV/7AAD staining. Cytokine concentration was assessed using ELISA.

Results: In the oxazolone induced colitis model, ITK-KO mice are protected against the development of intestinal inflammation compared to control mice. Upon administration of CsA there is an induction of apoptosis in LPMCs from control mice. Conditional NFATc1-ΔCD4-KO mice show no protection against oxazolone induced colitis compared to control mice. Interestingly, administration of CsA could not prevent inflammation in these mice.

Discussion / Conclusion: Our results indicate that in the oxazolone induced colitis model, CsA induces enhanced apoptosis in LPMCs of control- and ITK-KO mice. NFATc1 doesn’t seem to play a pivotal role in the development of intestinal inflammation since its knockout doesn’t lead to protection against the induced colitis even after administration of CsA. Therefore, we suggest ITK to be a possible target for the therapy of colitis ulcerosa.

P.C2.06.12
Optimization of nanobodies for in vivo targeting of P2X7 ion channel on brain microglia and kidney immune cells

C. Pinto-Espinosa1, N. Schwarz2, B. Rissiek1, M. Junge1, T. Magnus1, F. Hoag1, C. Stortelers1, F. Koch-Nolte1;
1University Medical Center Hamburg-Eppendorf, Hamburg, Germany, 2AbyIx NV, Ghent, Belgium.

Nanobodies are the smallest antigen-binding domains derived from heavy chain antibodies, naturally occurring in Camelds. The P2X7 channel is expressed by immune cells and promotes inflammatory responses. We generated nanobodies that effectively antagonize P2X7 and show benefit in animal models of inflammation (1). The clinical efficacy of these biologic antagonists is thought to reflect their excellent in vivo tissue penetration. However, little is known about the capacity of nanobodies to penetrate peripheral endothelial barriers or the highly restrictive blood-brain barrier (2). In this study, we reformatted P2X7 antagonistic nanobodies to improve tissue penetration. By genetic fusion, we dimerized them to increase avidity and added an albumin-specific domain. In vivo time penetration of the nanobodies was raised only in RA patients: CD4 FoxP3+ lymphocytes (r=-0.361;p=0.031). However, little is known about the capacity of nanobodies to penetrate peripheral endothelial barriers or the highly restrictive blood-brain barrier (2). In this study, we reformatted P2X7 antagonistic nanobodies to improve tissue penetration. By genetic fusion, we dimerized them to increase avidity and added an albumin-specific domain. In vivo time penetration of the nanobodies was raised only in RA patients: CD4 FoxP3+ lymphocytes (r=-0.361;p=0.031).

Discussion / Conclusion: Our results indicate that in the oxazolone induced colitis model, CsA induces enhanced apoptosis in LPMCs of control- and ITK-KO mice. NFATc1 doesn’t seem to play a pivotal role in the development of intestinal inflammation since its knockout doesn’t lead to protection against the induced colitis even after administration of CsA. Therefore, we suggest ITK to be a possible target for the therapy of colitis ulcerosa.

P.C2.06.13
Exploring the immunosuppressive potential of venom-derived molecules

R. Y. M. Ryan1,2, V. Luckwy1, J. Patriot1, M. Ionnamopoulou1, A. Lopez, J. J. Miles;1
1Australian Institute of Tropical Health and Medicine, Cairns, Australia, 2Griffith University, Brisbane, Australia, 3QIMR Berghofer, Brisbane, Australia, 4IMDEA Food Institute, Madrid, Spain.

The unique combinations of potent, specific, and fast-acting molecules within venom act to rapidly disrupt vital biological processes in prey and predators. Ironically, the same characteristics that make venom biologically effective presents an ideal platform for the exploitation of immunosuppressive pathways. This study mapped snake venom components with potent immune modulating abilities for drug development in the field of chronic inflammatory disease, of which many have no cure. Immunosuppressive venoms were fractionated using RP-HPLC and screened for activity against mitogen-induced activation. The effects of venom on human leukocytes were assessed using multiplex bead-based assays, flow cytometry, proliferation assays and cell viability assays. The results showed that specific venom fractions significantly inhibited IFNγ and TNFα release with primary leukocytes were stimulated with either Cell Stimulation Cocktail or CD3/CD28 activation beads. These results presented a new model for the exploitation of immunosuppressive venom components to use in response to lipopolysaccharide activation. Further, venom treatment reduced T-cell secreted cytokines without inhibiting cell proliferation or reducing cell viability, suggesting that these activation pathways are distinct in humans. Collectively, these data reveal novel venom-derived molecules specifically target and deactivate T-cells and could potentially be used to control or fine-tune the function of the human immune system.
Phenotypic changes of lymphocyte populations in psoriasiform dermatitis animal model

P. C2.06.18

K. Schinnerling1, C. Schäfer2, J. C. Aguillón2, V. Melissas5, A. Sarantopoulos1,2, R. Beyaert1, J. C. Aguillón2

1Universidad de Chile, Facultad de Medicina, Instituto de Ciencias Biomédicas, Santiago, Chile, 2Universidad San Sebastián, Departamento de Ciencias Morfológicas, Facultad de Ciencia, Santiago, Chile, 3Hospital Clínico Universidad de Chile, Sección de Reumatología, Santiago, Chile

Introduction: Preclinical testing of novel therapeutic approaches for rheumatoid arthritis (RA) requires a mouse model that imitates the human disease. To circumvent limitations due to differences between murine and human immune systems, we intended to establish a humanized mouse model of RA by transferring peripheral blood mononuclear cells (PBMCs) from healthy or RA subjects into immunodeficient mice. Methods: PBMCs were injected intravenously in 5–8-week-old female NOD-scid IL2γ−/− (NSG) mice. Human cell engraftment was monitored weekly and reactivity of human cells, recovered from spleens, towards synovial fluid (SF) and polyclonal stimulation, was assessed by flow cytometry. Human immunoglobulins (Ig) were quantified in serum by ELISA. Joint sections were stained with H&E. Results: Engraftment of human cells reached 36±5% in spleen, 77±4% in lymph nodes, 62% in bone marrow and 22±4% in blood of NSG mice at six weeks after PBMC injection, regardless of whether PBMC from healthy or RA donors were used. The human graft consisted predominantly of activated CD4+ T cells which preserved their capacity to express IFN-γ in response to SF or polyclonal stimulation. Engrafted B cells continued producing IgM, IgG and IgE. Injection of RA patient-derived PBMC alone failed to generate histological alterations, however, in pilot experiments, additional intrarticular administration of SF, or injection of RA-derived CD4+ T cells together with SF-pulsed dendritic cells, resulted in cellular infiltrates and cartilage degradation. Conclusion: Although requiring further investigation, this chimeric human mouse model of RA might result in an experimental system for preclinical testing of new therapies. Funded by FONDECYT3150453 and FONDECYT1140553.

P. C2.06.17

Characterization of MAL1T protease resistant CYLD knock-in and HOIL-1 knock-out mice

I. Skordov1, A. Demery2, J. Staaf3, R. Beyaert1

1VIB-Ugent Center for Inflammation Research, Ghent (zwaardebouw), Belgium, 2Department of Biomedical Molecular Biology, Ghent University, Ghent (zwaardebouw), Belgium

Introduction: The parasapace MAL1T is a key molecule in TCR induced signaling to NF-κB and steers TCR-induced gene expression by two different mechanisms: as a protease and as a scaffold protein. MAL1T proteolytic activity fine-tunes inflammatory gene expression and contributes to antigen-induced T cell proliferation as revealed by studies on MAL1T protease deficient (MAL1T-PD) mice. Remarkably, MAL1T-PD mice suffer from ataxia and lethal multi-organ inflammation, have reduced frequencies of thymic and peripheral regulatory T cells (Tregs and pTregs) and marginal zone (MZ) B cells, but have increased frequencies of effector T cells. Here, we investigated whether preventing the cleavage of an individual MAL1T substrate, CYLD or HOIL-1, leads to a similar phenotype. Materials and Methods: CYLD knock-in (KI) and HOIL-1 KI mice, expressing a non-cleavable form of CYLD as a scaffold protein. MALT1 proteolytic activity fine-tunes inflammatory gene expression and contributes to antigen-induced T cell proliferation as revealed by studies on MALT1-PD mice. Results and conclusions: In contrast to MALT1-PD mice, our KI mice did not develop ataxia or multi-organ inflammation. In addition, frequencies of Tregs, pTregs, MZ B cells, Th1, Th2, Th17 and Tc1 were comparable between KI and wild-type mice as were T cell activation status, the frequencies of tTregs, pTregs, MZ B cells, Th1, Th2, Th17 and Tc1 were comparable between KI and wild-type mice. The main statistically significant changes in the respective intracellular signaling pathway.

P. C2.06.16

Establishment of a chimeric human-mouse model of rheumatoid arthritis for pre-clinical testing

K. Schinnerling1, C. Schäfer2, J. C. Aguillón2, V. Melissas5, A. Sarantopoulos1,2, R. Beyaert1, J. C. Aguillón2

1Universidad de Chile, Facultad de Medicina, Instituto de Ciencias Biomédicas, Santiago, Chile, 2Universidad San Sebastián, Departamento de Ciencias Morfológicas, Facultad de Ciencia, Santiago, Chile, 3Hospital Clínico Universidad de Chile, Sección de Reumatología, Santiago, Chile

Introduction: Preclinical testing of novel therapeutic approaches for rheumatoid arthritis (RA) requires a mouse model that imitates the human disease. To circumvent limitations due to differences between murine and human immune systems, we intended to establish a humanized mouse model of RA by transferring peripheral blood mononuclear cells (PBMCs) from healthy or RA subjects into immunodeficient mice. Methods: PBMCs were injected intravenously in 5–8-week-old female NOD-scid IL2γ−/− (NSG) mice. Human cell engraftment was monitored weekly and reactivity of human cells, recovered from spleens, towards synovial fluid (SF) and polyclonal stimulation, was assessed by flow cytometry. Human immunoglobulins (Ig) were quantified in serum by ELISA. Joint sections were stained with H&E. Results: Engraftment of human cells reached 36±5% in spleen, 77±4% in lymph nodes, 62% in bone marrow and 22±4% in blood of NSG mice at six weeks after PBMC injection, regardless of whether PBMC from healthy or RA donors were used. The human graft consisted predominantly of activated CD4+ T cells which preserved their capacity to express IFN-γ in response to SF or polyclonal stimulation. Engrafted B cells continued producing IgM, IgG and IgE. Injection of RA patient-derived PBMC alone failed to generate histological alterations, however, in pilot experiments, additional intrarticular administration of SF, or injection of RA-derived CD4+ T cells together with SF-pulsed dendritic cells, resulted in cellular infiltrates and cartilage degradation. Conclusion: Although requiring further investigation, this chimeric human mouse model of RA might result in an experimental system for preclinical testing of new therapies. Funded by FONDECYT3150453 and FONDECYT1140553.

P. C2.06.15

Impact of rituximab therapy on IgG4 levels and B-cell phenotype in rheumatoid arthritis patients

A. Sarantopoulou1, E. Farmaki1, M. Mytilinou1, A. Glantrakos1, A. Sarantopoulou1, P. Boura1, A. Tsirigioti1

12nd Department of Internal Medicine, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece, 2First Department of Pediatrics, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece, 3Department of Immunology and Histocompatibility, ‘Evangelismos’ General Hospital, Athens, Greece

Introduction: Rituximab is an effective therapeutic option in a variety of diseases. On IgG4-RD, apart from B-cell depletion, rituximab induces remission by reducing IgG4 levels. On a previous study has inter-associated IgG4 levels and B-cell phenotype following rituximab therapy. We investigated whether B-cell depletion in RA: i) induces selective reduction of IgG4, ii) IgG4 alterations follow specific B-cell immunophenotype imprint. Patients and Methods: 31 RA patients, on a standard of care DMARD treatment and rituximab administration every 6 months for 2 years were investigated for alterations on disease activity along with IgG subclasses levels and B-cell immune profile. All parameters were assessed at enrollment (T0), and after 6, 12 and 24 months. On this 2-year period, all patients had been periodically receiving rituximab every 6 months. Results: After 2 years of rituximab therapy, 4 patients had achieved a remission. IgG4 levels though not statistically declined. Special features of emerging B-cell subpopulations have been identified in selected patients. Conclusions: This is the first time that IgG4 variations and B-cell emerging subpopulations are co-investigated in a non-IgG4RD after rituximab administration. Our results imply that IgG4 may be actively implicated in RA pathophysiology since disease remission is accompanied by only IgG4 level reduction among all classes and subclasses of IgG. These findings open a new chapter in RA pathophysiology, indicating that this subclass is actively participating in the homeostasis of immune-mediated diseases other than IgG4-RD.

P. C2.06.14

Phenotypic analysis of a protective for rheumatoid arthritis TRAF-1 SNP

A. Sarantopoulou1, K. Papavasileiou1, G. Leonis1, V. Melissar1, J. Theodorou1, P. Boura1, A. Notopoulos1

12nd Department of Internal Medicine, Hippokration General Hospital, Aristotle University of Thessaloniki, Greece, 2Department of Chemistry and Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece, 3Department of Chemistry, University of Ioannina, Ioannina, Greece, 4Institute of Nanosciences and Nanotechnology, National Centre for Scientific Research "Demokritos", Athens, Greece

Introduction: Immunogenetic studies in Rheumatoid Arthritis (RA) have documented the positive correlation of various gene loci with incidence and/or disease profile. However, the description of gene loci negatively related to the incidence of RA is rarely documented. Even more seldom is the functional, proteomic analysis of such an SNP and of the respective intracellular signaling pathway. Methodology - Results: We performed the immunogenetic sequence of the seven exons of the gene TRAF1. 172 patients and 95 controls were genetically assessed. On the position 9:120905076 of exon 7, the registered polymorphism G/A (rs143326508) was described in the controls group. The same polymorphism was not confirmed in any of the patients. Further functional proteomic study of the polymorphism with computing programs (software), revealed that the presence of this polymorphism leads to a differentiation of the quaternary structure of TRAF1 protein. A further three-dimensional configuration of the TRAF1 associated molecules was performed in order to assess the signaling deviation this SNP may elicit on the TRAF1 signaling cellular pathway. Presentations: The current presentation is one of the extremely rare genetic studies describing a protective gene locus against rheumatoid arthritis, and a pioneer of its kind in the use of Applied Informatics in the depiction of the quaternary structure of the encoded protein. At the same time, it is one of the few immunogenetic studies describing the functional proteomics of the encoded protein, plotting on a molecular level specific interaction modifications affecting the intracellular signaling pathway of TNFα.

P. C2.06.13

Increase of erythema, scaling, thickening values and PASI score were registered; histology confirmed psoriasiform dermatitis. The main statistically significant changes in the respective intracellular signaling pathway.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.C2.06.19
Bone marrow derived mesenchymal stromal cells suppress Th1 and enhance Th17 differentiation of antigen-activated CD4+ T cells in vitro
J. L. Talbot1, L. S. Börsen2, M. R. von Essen3, A. Fischer-Nielsen4, R. S. Oliver5, M. Blenkinsop6, F. Sellebjerg7,8
1Danish Multiple Sclerosis Center, Copenhagen, Denmark, 2Danish Multiple Sclerosis Center, Copenhagen, Denmark.
Mesenchymal stromal cells (MSCs) can modulate immune cell proliferation, activation and differentiation. The aim of this study was to assess how MSCs regulate the proliferation and differentiation of healthy immune cells in response to specific antigens known to induce different T helper cell (Th) responses. Peripheral blood mononuclear cells (PBMCs) from healthy individuals (n=13) were incubated with bone-marrow derived MSCs from 1 MS patient (n=1) and 1 healthy donor (n=1). PBMCs were cultured with six different antigens for 7 days and analyzed by flow cytometry. The expression of the chemokine receptors CCR3 and CCR5 were analyzed on CFSE- labeled T cells as surrogate markers for specific Th responses. Furthermore, we analyzed a panel of intracellular cytokines in CD8+ T cells derived from healthy donors and infected with MSCs. Stimulation of PBMCs in coculture with MSCs. Stimulation of CD4+ T cells was significantly diminished when stimulated with antithrombin responses. The suppression of Th17- and Th1-associated chemokine receptor in CD8+ T cell responses was significantly enhanced, respectively, by mesenchymal stromal components. Intracellular analysis revealed significantly increased expression of Th17 cytokines and decreased expression of Th1 cytokines on CD8+ T cells cultured with bone marrow stromal cells.
Our results indicate that bone marrow stromal cell-induced suppression of CD4+ T cell proliferation may depend on the type of Th response. Stromal cells can suppress Th1-related responses and enhance Th17-related responses in CD4+ T cells in a dose-response dependent manner.

P.C2.07.01
Immune signaling and therapy in autoimmunity - Part 7

P.C2.07.02
Protective antibodies against rheumatoid arthritis
B. Xu, L. Liang1, O. Tong1, E. Lönndahl1, C. Gei2, A. Olsson3, H. Hofström4, L. Håhlman5, W. Cai6, R. Holmdahl7

Introduction: Rheumatoid arthritis (RA) is a common chronic inflammatory disease that affects mainly peripheral joints. Sadly here is no cure for RA yet. However, our earlier research found some of the defined epitopes located on Collagen II seem to be associated with protection against arthritis.

Materials and methods: Arthritis is induced in 3-4 months old male by intravenous injection of pathogenic monoclonal antibody (mAb) cocktail. One group received PBS and the others received antibodies to the certain epitopes respectively one day after injection of arthritogenic antibodies. All mice were boosted with 50µg Endotoxin (lipopolysaccharide) by intraperitoneal injection on day 5. Arthritis was scored every second day until day 21 post immunization.

Results: Antibodies to this particular epitope could suppress the development of arthritis if injected shortly after injection of arthritogenic antibodies.

Conclusion: Antibodies to the certain epitope protects against arthritis in mice.

P.C2.07.03
The role of anticytokine autoantibodies in the pathogenesis of juvenile idiopathic arthritis
Y. Chen1,2, Y. Yang1, B. Chiang1
1Department of pediatrics, Taipei City United Hospital, Taipei, Taiwan, 2Department of pediatrics, National Taiwan University Hospital, Taipei, Taiwan.

Introduction: anti-cytokine autoantibodies (ACA) have been reported in healthy people and patients with a variety of autoimmune diseases, but their function is still debated.

There is little study about ACA in juvenile idiopathic arthritis (JIA). The purpose of study is to examine anti-cytokine antibodies in the patients with juvenile idiopathic arthritis patients. Material and methods: Fifty seven patients in whom oligoarticular, polyarticular and systemic type JIA were diagnosed before 16 years old were recruited on visits to the pediatric rheumatology department (from 2011 to 2015) and twenty healthy control were recruited. The ACA was assayed by indirect ELISA for anti-TNF alpha, anti IL-6, anti IL-10 autoantibodies. Results: Anti-TNF alpha IgG and IgM show significantly higher level in all subtype JIA group than control group (P<0.05). Anti IL-6 IgG and IgM level showed significant higher in all subtype JIA group than control group (P<0.01). Anti IL-6 IgG showed higher level in active disease (CHAQ>0) than inactive disease (CHAQ=0) in oligoarticular and polyarticular subtypes. The anti IL-6 IgG level showed positive correlation with WBC, PLT, ESR, CRP in systemic subtype. Conclusions: Anti-TNF alpha and anti IL-6 autoantibodies are higher in Juvenile idiopathic arthritis than those of healthy control in some subtypes. Although systemic type JIA is considered as autoinflammatory disease, we found autoantibodies in these patients. These antibodies might play an important role in pathologic mechanism, disease modulation or just reflect the increasing serum level of these cytokines.

P.C2.07.04
Protective effects of mixture of fifteen n-propyl polysulfides on ConA-induced hepatitis mediated by induction of regulatory macrophages

1Faculty of Medical Sciences, Kragujevac, Serbia, 2Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia, 3Card center for New Technologies, Belgrade, Serbia, 4Faculty of Chemistry, Belgrade, Serbia.

Biologically active substances of garlic are different organosulfur compounds that can exhibit potent antioxidant, anti-inflammatory, immunomodulatory and antitumor activities. The biological activity of organosulfur compounds is in direct correlation with the number of sulfur atoms. ConA induced hepatitis is acute form of inflammatory liver disease that shares some features of medullary mediated liver diseases, including autoimmune hepatitis. In this study, immunomodulatory and antiinflammatory potential of the mixture of fifteen n-propyl polysulfides was analysed in ConA-induced hepatitis in mice. Mixture of fifteen n-propyl polysulfides was orally administered to C57BL/6 mice eight hours before intravenous injection of ConA. Disease severity was evaluated by liver enzyme assay, quantitative histology, mononuclear cell infiltration, cytokine production, liver endothelial cell activation and percentage of hepatocellular apoptosis. Mixture of fifteen n-propyl polysulfides almost completely prevented damage of liver tissue, attenuated production of inflammatory cytokines and enhanced infiltration of liver tissue with activated regulatory (CD68+CD16+H-144+) macrophages and regulatory T cells. In conclusion, mixture of n-propyl polysulfides exerts highly antinflammatory and immunomodulatory activity. This mixture enhances liver infiltration with regulatory macrophages and thus prevents ConA induced liver damage.

P.C2.07.05
Insufficient interleukin-10 production as a mechanism underlying pathogenesis of systemic juvenile idiopathic arthritis
M. Imbrechts1, A. Avanu2, J. Vandenhaute2, B. Maleniger-Delvies3, K. Puri4, T. Mitter5, N. Berghmans6, O. Burton7, A. Lisson8, Lis de Somer9, C. Wouters10, M. Mathys10
1Rega Institute, KU Leuven, Leuven, Belgium, 2VIB-KU Leuven, Leuven, Belgium, 3University Hospital Leuven, Leuven, Belgium.

Objective: Systemic juvenile idiopathic arthritis (sJIA) is a childhood immune-inflammatory disorder with unknown etiology. One of the concepts is that the disease results from an inappropriate control of immune responses to an initially harmless trigger. We investigated whether sJIA may be caused by defects in IL-10, a key cytokine in controlling inflammatory responses. Methods: IL-10 production was analyzed in a sJIA mouse model, which relies on injection of Complete Freund’s Adjuvant (CFA) in INF-γ deficient mice. Corresponding wild type (WT) mice develop a subtle and transient inflammatory reaction and were used to study the effect of IL10 neutralization. Cytokines and CRP were analyzed in plasma of sJIA patients (active: n=10; inactive: n=8) and healthy controls (n=15). Their PBMCs were used to study cell-specific defects in IL-10.
POSTER PRESENTATIONS

Results. Diseased IFN-γ deficient mice showed a defective IL-10 production in T<sub>reg</sub> cells, B cells and NK cells, with B cells as the major source of IL-10. Neutralization of IL-10 in WT mice restored immune-inflammation but did not consistently ameliorate of sita. In sIa patients, IL-10 positive levels were strikingly low as compared to pro-inflammatory mediators. In addition, B cells from sIa patients showed a decreased IL-10 production, both ex vivo and after stimulation.

Conclusion. Cell-specific IL-10 defects in sIa mice and patients result in an insufficient IL10 production to counterbalance their pro-inflammatory cytokines. IL-10 neutralization in CIA-challenged WT mice converts a transient inflammatory reaction into a chronic disease and represents a model for sIa in IFN-γ-deficient mice.

P.C2.07.06

Exploring the role of genetic variants in gene expression in the context of chronic inflammation

S. Koturan<sup>1,2</sup>, S. Menegatti<sup>1</sup>, E. Latsi<sup>1,2</sup>, N. Rosine<sup>1,2</sup>, H. Yoha<sup>1</sup>, C. Miceli-Richard<sup>1,3</sup>, E. Blancho<sup>1</sup>, L. Roggeri<sup>1</sup>,<sup>1</sup>Centro Nazionale di Ricerca Rodriguez Pignatelli, Gif sur Yvette, France; <sup>2</sup>University Paris Diderot, Ecole Doctorale BioGCP, Paris, France; <sup>3</sup>Immunological Research in Spondyloarthritides (IRS), Unité Mixte de Recherche, Department of Immunology, Institut Pasteur, Paris, France, Service de Rhumatologie, Hôpital Cochin, Paris, France.

Introduction: Genome-wide association studies have provided detailed information about the genetic variants associated with chronic inflammatory diseases. However, for most genetic variants, the mechanism by which they act and the targeted cell populations are unknown. The general goal of this study is to decipher how SNPs associated with chronic inflammatory disease affect pathogenesis. In particular, we will determine which genetic variants affect cell type-specific gene expression using axial spondyloarthritis (SpA) as a model.

Materials and Methods: Whole blood samples from patients were cultured with a microbial stimulus (LPS) or a T-cell agonist (enterotixin SEB). CD14<sup>+</sup> monocytes were isolated using magnetic bead selection from PBMCs followed by LPS stimulation. CD4<sup>+</sup> and CD8<sup>+</sup> T-cell populations were isolated by cell sorting and stimulated with anti CD3/CD28. We designed a gene expression panel that allows us to measure the expression of 755 genes that are either associated with chronic inflammatory diseases or the regulation of immune response. Patients were grouped using the HMDB<sup>+</sup> database.

Results: Of the 755 genes analysed, around 600 genes were expressed in both LPS stimulated and SEB stimulated whole blood samples. For monocytes, we detected expression of 250 genes after stimulation. Our eQTL analysis revealed associations that regulate several immune-modulatory pathway-associated genes. The functional relevance of these findings warrants further study.

Conclusions: Our study demonstrates that the majority of genes genetically linked to chronic inflammatory diseases are expressed in whole blood and immune cell populations from SpA patients pointing to new insights in pathogenesis of chronic inflammatory diseases.

P.C2.07.07

Comparative analysis between the in vivo biodistribution and therapeutic efficacy of adipo-derived mesenchymal stromal cells administered intraoperatively in experimental colitis

M. Lopez-Santalla<sup>1</sup>, P. Mancheño-Corvo<sup>1</sup>, A. Escalon<sup>1</sup>, R. Ment<sup>1</sup>, O. Delarosa<sup>1</sup>, J. Abad<sup>1</sup>, D. Büscher<sup>1</sup>, J. M. Redondo<sup>2</sup>, J. A. Buener<sup>1</sup>, W. Daleman<sup>1</sup>, E. Lombardo<sup>1</sup>, M. Garin<sup>1</sup>,<sup>1</sup>CENMI Fundación Jiménez Diaz/CIBERER, Madrid, Spain; <sup>2</sup>Tigenix SAU, Madrid, Spain; <sup>3</sup>The Rockefeller University, New York City, United States, <sup>4</sup>Céretrop$a$, Madrid, Spain; <sup>5</sup>Grifols, Barcelona, Spain; <sup>6</sup>Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; <sup>7</sup>Tigenix NV, Leuven, Belgium.

INTRODUCTION: Mesenchymal stem cells (MSCs) have immunomodulatory properties allowing their use for treatment of a wide variety of immunological disorders. The mechanisms that are involved in their therapeutic effects have not been fully determined. Immunomodulatory effect of MSCs takes place both by direct cell-to-cell contact and by soluble factors. A small proportion of infused MSCs can traffic to the draining lymph nodes accompanied with an increase of different types of regulatory immune cells. Intraoperative injection of MSCs is being used in the clinic for treatment of cancer and allergy and can be an alternative route for MSC administration.

METHODS: We analyzed the biodistribution and the efficacy of Luciferase<sup>+</sup> adipo-derived MSCs (Luci-eASCs), infused through the inguinal LNs (ILNs), in an experimental mouse model of TNBS-induced colitis.

RESULTS: Luci-eASCs were able to modulate the acute inflammatory response induced by the administration of TNBS. The large majority of Luci-eASCs were found in the ILNs and in the adipose tissue surrounding the injection site. Increase bioluminescence signal was found in the intestine of colitic mice compared to healthy controls. Luci-eASC-infused mice were stratified according to their positive response to the Luci-eASC treatment. A higher accumulation of Luci-eASCs was found in popliteal, parathymic, parathyroid and mesenteric ILNs in those mice that had a positive response to Luci-eASCs.

CONCLUSIONS: Acute intestinal inflammatory responses can be modulated by intraoperational administration of Luci-eASCs. The accumulation of the eASCs at the inflamed intestine may be beneficial to achieve an optimal modulation of inflammation following intraoperational administration.

P.C2.07.08

In vivo biodistribution of adipose derived mesenchymal stromal cells administered intraperitoneally in experimental colitis

M. Lopez-Santalla<sup>1</sup>, P. Mancheño-Corvo<sup>1</sup>, A. Escalon<sup>1</sup>, R. Ment<sup>1</sup>, O. Delarosa<sup>1</sup>, J. M. Redondo<sup>2</sup>, J. A. Buener<sup>1</sup>, W. Daleman<sup>1</sup>, E. Lombardo<sup>1</sup>, M. Garin<sup>1</sup>,<sup>1</sup>CENMI Fundación Jiménez Diaz/CIBERER, Madrid, Spain; <sup>2</sup>Tigenix SAU, Madrid, Spain; <sup>3</sup>The Rockefeller University, New York City, United States, <sup>4</sup>Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; <sup>5</sup>Tigenix NV, Leuven, Belgium.

INTRODUCTION: The use of mesenchymal stem cells (MSCs) in the clinical field has gathered tremendous momentum over the last decade due to the varying levels of success in treatment of inflammatory and autoimmune diseases thanks to their immunomodulatory properties. The immunomodulatory effect of MSCs takes place both by direct cell-to-cell contact and by soluble factors. Although the biodistribution of MSCs highly depends on the route of administration, similar efficacy has been described regardless the administration route used. Several studies have shown that a small proportion of the infused MSCs can be found at the site of inflammation. At present, it is unknown whether the migration of the MSCs to the inflamed tissues is a prerequisite to achieve their beneficial effect.

METHODS: We analyzed the biodistribution of intraperitoneally (IP) administered luciferase expressing human expanded adipose derived stem cells (Luci-eASCs), in a mouse model of colitis. Stratification of mouse responses to the Luci-eASC treatment allows us to define whether a correlation between the biodistribution of Luci-eASCs and the therapeutic efficacy exists.

RESULTS: IP administered Luci-eASCs were mainly found in the liver, spleen and intestine. In the intestine of colitic mice, a higher accumulation of Luci-eASCs was found, compared to healthy controls. The bioluminescence signal in the intestine tended to increase at the expense of a decrease in the liver in the ‘responder’ mice.

CONCLUSIONS: These data thus suggest that the accumulation of the eASCs to the inflamed tissues is beneficial in order to achieve modulation of the inflammatory insult.

P.C2.07.09

Peptide-specificity of the CD4 T-cell response to immunogenic therapeutic antibodies in healthy donors and in patients.

S. Meunier, M. de Bourayne, M. Hamze, B. Mailleire,
C.EA, GiF sur Yvette, France.

Immunogenicity of therapeutic antibodies is an important limitation to their clinical use. Infliximab (IFX), Rituximab (Rtx), Adalimumab (Adm) and Natalizumab (Nzt) are all known to induce neutralizing anti-drug antibodies (ADA) in many patients. Because CD4 T-cells initiate immune responses, we identified the CD4 T-cell epitopes of these antibodies. We hypothesized that immunogenicity of therapeutic antibodies that are immunogenic in healthy donors is also immunogenic in patients. CD4 T-cells were expanded by several weekly rounds of antigen-specific stimulation and the T-cell specificity was assessed by ELISPOT using overlapping peptides. Nine epitopes were identified in the VL and VH chains of chimeric Rtx and IFx and overlapped CDR or FR regions. Nine and eleven T-cell epitopes were found in the humanized and human antibodies Nzt and Adm, respectively. Several peptides were common to multiple donors and their location appeared to rely on their affinity for HLA molecules and their content in mutations with respect human germline sequences. T-cell reactivity of the identified peptides was evaluated in patients having developed (ADA+) or not developed ADA (ADA−). Two third of the T-cell epitopes of Rtx and IFx identified from the healthy donors stimulated PBMCs from ADA+ patients, while T cell reactivity of Adm T-cell epitopes is higher in ADA+ patients than in ADA-. T-cell epitopes promote the secretion of a diversity of cytokines either favoring or reducing ADA production. Together our data provide new insights on the origin and mechanisms of immunogenicity of therapeutic antibodies.

P.C2.07.10

A CD4 T-cell repertoire specific to immunogenic self therapeutic proteins exists in healthy donors before any injection

S. Meunier, A. Azem, M. de Bourayne, B. Mailleire,
C.EA, GiF sur Yvette, France.

Many hormones, cytokines and clotting factors are used as therapeutic molecules but many of them produce anti-drug antibodies (ADA), although their peptide sequence is of human origin. To quantify the rare antigen-specific T-cells from healthy donors, who have never been exposed to the antigen, CD4 T-cells were stimulated by weekly rounds of stimulation by antigen-loaded dendritic cells and their specificity was assessed by IFN-γ ELISPOT. The number of specific T-cell lines was used to estimate the frequency of circulating human T-cells specific to IFN-β and demonstrated the T cell epitopes spread all over the IFN-β sequence, two regions being commonly recognized by ADA+ and ADA− patients. H2-restricting with a insulin-like structure and has been found to induce diabetes in streptozotocin diabetic patients.
A very large repertoire of T cells specific for H2-Relaxin was found in the healthy donors. We also identified two major T-cell epitopes hosted in the a and b chains and common to multiple donors. The frequency of SVIL-specific CD4 T cells in healthy donors was very high and similar to that of T cells specific for foreign antigens. We also observed that SVIL-specific T cells originated from both the naive and central memory cells. Altogether our results showed that endogenous expression of IFN-β SVIL and H2-Relaxin is not sufficient to prevent an escape of CD4 T cells from negative thymic selection and to abrogate immunogenicity of the recombinant forms.

P.C2.07.11
IgG and IgM play a main role in the development of demyelinating lesions and axonal damage in multiple sclerosis.

U. Mukhó 1, C. Sédé 1, E. Escudero 2, M. M. Eslín 1, I. Iturrieta 1, C. Sloan 1, A. Joyo 1, M. C. Sádaba 1

Introduction. Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by demyelination and axonal damage. We have described that IgG and IgM co-localized with complement and macrophages on oligodendrocytes and axons. Oxidative stress is also a hallmark of the lesions. Therefore, we aimed to analyze the role of antibodies and oxidative stress in these patients.

Materials and Methods: Brain samples from patients suffering from MS, neurodegenerative diseases (Alzheimer, Parkinson, Frontotemporal Dementia (FTD), amyotrophic lateral sclerosis (ALS)), and control group. Immunohistochemistry and immunofluorescence techniques were used to detect IgG, IgM, oxidative stress (ES6), axonal damage (amyloid precursor protein, APP). TUNEL assay was used to detect cell death.

Results. ES6, IgG and IgM were not detected in samples from patients with Alzheimer, Parkinson or control group. In samples from MS patients, IgG and IgM were observed on T-glia and an infiltration of inflammatory cells with impaired body weight gain, colon length, ratio of colon weight to body weight, disease activity index and histopathological scores of MCC950 treated Winnie mice were significantly reduced suggesting not only attenuation of ongoing colitis but also delay of disease onset. MCC950 significantly suppressed IL-1β and IL-18 cytokine expression at both mRNA and protein levels in Winnie colons.

Additionally, MCC950 also effectively suppressed the release of proinflammatory cytokines (IL-1α, IL-17, TNF-α and IFNγ) and chemokine (MIP1α) in mucosal explants. Moreover, MCC950 treatment resulted in a significant decrease of IL-1β release and activation of caspase-1 in Winnie explants and in vitro macrophage cells isolated from Winnie mice. The treatment of 10µM MCC950 in Winnie mucosal explants shows, for the first time, the contribution of anti-inflammatory effects resulting exclusively from inhibition of canonical and non-canonical NLRP3 inflammasome activation in colitis. Taken together, our results illustrate the efficacy of MCC950 in the treatment of murine ulcerative colitis and provides novel potential targets for IBD treatment in humans. Work was supported by SF6863 and NE14662-2 grant from DFG (S.N.), and Russian Science Foundation grant #14-50-00060 (S.N.) and # 17-74-20059 (A.K.).

P.C2.07.13
MCC950 attenuates colonic inflammation in spontaneous colitis mice

A. P. Perera, R. Eri
Shool of Health Sciences, Launceston, Australia.

MCC950 is a potent, highly specific small molecule inhibitor of both canonical and noncanonical activation of NLRP3 inflammasome and has been evaluated in a diverse array of NLRP3 engaged inflammatory diseases. However, the effect of MCC950 on colitis has not yet been reported. In the present study we investigated the effect of MCC950 in a spontaneous chronic colitis mouse model Winnie, which mimics human ulcerative colitis. Oral administration of 40 mg/kg MCC950 for three weeks at chronic stage of colitis significantly alleviated colitis with improved body weight gain, colon length, ratio of colon weight to body weight, disease activity index and histopathological scores. MCC950 treated Winnie mice were observed to show reduced inflammatory cell infiltration in colon and colons of mice treated with MCC950 showed reduced IL-1β and IL-18 cytokine expression at both mRNA and protein levels in Winnie colons.

Additionally, MCC950 also effectively suppressed the release of proinflammatory cytokines (IL-1α, IL-17, TNF-α and IFNγ) and chemokine (MIP1α) in mucosal explants. Moreover, MCC950 treatment resulted in a significant decrease of IL-1β release and activation of caspase-1 in Winnie explants and in vitro macrophage cells isolated from Winnie mice. The treatment of 10µM MCC950 in Winnie mucosal explants shows, for the first time, the contribution of anti-inflammatory effects resulting exclusively from inhibition of canonical and non-canonical NLRP3 inflammasome activation in colitis. Taken together, our results illustrate the efficacy of MCC950 in the treatment of murine ulcerative colitis and provides a novel therapeutic strategy for human inflammatory bowel diseases.

P.C2.07.14
Effect of Tripterygium wilfordii polypolyon on inflammatory pathology and TL44 signaling pathway in ulcerative colitis rats model

D. Qin 1, Y. Zhou 1, G. Yang 1, C. Zhang 1, S. Zhang 2, Q. Dai 1, X. Yang 1
1The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China, 2The First Clinical Medical College of Zhejiang Chinese Medical University, Hangzhou, China.

Tripterygium wilfordii polypolyon (TCP) is a water-chloroform extract from a kind of Chinese medicine-Tripterygium wilfordii Hook. f. It preserves a good immune regulation effect and has been applied to the treatment of some autoimmune diseases. However, no related studies on its role in ulcerative colitis (UC) were reported till now. In this work, we investigated the effect of TCP on UC inflammation and toll-like receptor 4 (TLR4) signaling pathway in TNBS-alcoholic UC rats model. The study found that TCP can significantly improve the symptoms of UC rats model and has a good inhibitory effect on intestinal inflammation. It not only promotes the healing of mucosal defects, but also inhibits the infiltration of inflammatory cells in mucosal lesions to acquire mucosal healing. Whereas, these effects are dose-dependent. On the research of its mechanism of action, we found that TCP exerts anti-inflammatory effects through inhibiting the protein expression of TLR4/MyD88-dependent and non-dependent signaling pathways, thereby inhibiting the release of downstream inflammatory factors.

P.C2.07.15
Clinical study on the effect of tripterygium wilfordii polypolyon on intestinal inflammation in inflammatory bowel disease

D. Qin 1, Y. Wang 1, G. Ni 1, C. Zhang 1, Y. Mao 1, G. Fang 1, X. Xu 1, H. Guo 2, Q. Liao 1, S. Zhu 1, Y. Yang 1, G. Cen 1
1The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China, 2The First Clinical Medical College of Zhejiang Chinese Medical University, Hangzhou, China.

Objective: To observe the effect of TCP on intestinal inflammation in IBD patients. Method: 32 IBD patients, with steroid hormones resistance or dependence or taking other immunosuppressive agents with tolerable side effects, were selected. We gave TCP oral treatment (60mg/d) on the basis of the original aminosalicylic acid treatment (4g/d).After treated for 24 months, we made a before and after treatment comparison analysis for patients, including clinical manifestations, endoscopic mucosal lesions, tissue inflammation, blood changes, mucosal healing, immune response and toxic side effects. Result: (1) TCP improve the symptoms of IBD patients significantly. (2)The scores of endoscopic MES and SES-CD were significantly decreased after TCP treatment. (3)After treatment, the score of mucosal inflammation injury in IBD patients is decreased. (4) The levels of ESR, CRP, PLT, HGB,ALB are improved.(5)25 patients have a complete response to TCP after treatment, partial response are 7 cases; (6) Hormone dependents included in this study is successfully discontinued after taking TCP 12 weeks and have a complete response to TCP treatment;(7) The major toxic side effects include gastrointestinal suppression,mild bone marrow suppression and mild liver damage in the study, but they will turn back to normal after reducing the dosage of TCP.Conclusion: TCP can relieve the clinical manifestation of IBD, inhibit intestinal inflammatory activity and acquire mucosal healing. Meanwhile, TCP can help the steroid hormone dependent patients to reduce the dosage of hormone smoothly until discontinuation, and to get the response and remission to the treatment.
Introduction: Chronic inflammatory diseases therapy has been revolutionized since the introduction of three main anti-TNFα drugs (Infliximab, Adalimumab and Etanercept). However, as most genetically engineered proteins, they are immunogenic, inducing the production of anti-drug antibodies (ADA) that lead to drug failure. The aim of our study is to assess the immunogenicity of these 3 anti-TNFα therapies. Materials and methods: Our study involved 42 patients (19 females and 23 males, mean age 38±13 years, disease duration 6±3 years), with established Rheumatoid arthritis (RA), Crohn disease or ankylosing spondylitis. All patients presented primary or secondary therapeutic failure after anti-TNFα use. Both drug and ADA levels were measured in patient’s sera using an enzyme-linked immuno sorbent assay (ELISA) [Promonitor®Progenika Biopharma SA, Spain]. Results: 29% of patients produced ADAs (IFX 36%, ADA 32% and ETN 0%). The presence of ADAs is associated with low drug levels below therapeutic range (>80%). Females (58%) and older patients (60%) are most likely to produce ADAs following a few number of injections in IFX patients vs. ADA and in females vs. males. However, the association of ADAs with reduced drug levels decreases significantly its immunogenicity. ADA frequency varies with the underlying disease but no significant influence of a previous biotherapy switch has been demonstrated in our study. Conclusion: Drug failure to anti-TNFα therapies is due to ADAs production. This immunogenicity is influenced by numerous factors that are either, drug related (drug structure, association to an immunosuppressant agent) or patient related (gender, age and the underlying disease).

TARGET CELLS OF VEDOLIZUMAB IN PERIPHERAL BLOOD AND GUT MUCOSA CELLS FROM IBD PATIENTS

W. T. C. Unikere Venema, M. D. Voskul, A. Bangma, B. H. Jansen, G. Dijkstra, R. K. Weersma, E. A. Festen; University Medical Center Groningen, Groningen, Netherlands.

Introduction: The biological vedolizumab (anti-α4β7) blocks the migration of leukocytes into the gut. Treatment results in remission in ~30% of Crohn’s disease (CD) and ulcerative colitis (UC) patients. In UC, remission is reached faster than in CD. Deeper insights into the immunological effects of vedolizumab necessary to explain differences in effect of vedolizumab therapy between CD and UC. Aims and Methods: The aim of this study is to determine the binding capacity of vedolizumab to both immune cells in blood and to isolated mucosal cells from the inflamed gut mucosa. We collected blood and intestinal biopsies from patients with CD and UC prior to vedolizumab treatment, and blood from healthy controls. We engineered fluorescent-labeled vedolizumab and assessed the percentage and level of vedolizumab-binding to cell-subtypes, using flow cytometry. Results: Vedolizumab binds to a variety of peripheral blood immune cells (i.e. CD4+T-cells, CD8+ T-cells, eosinophils, NK-cells and monocytes). It nearly covers all gut mucosa directed CD4+CD38+CD62L+ T cells (median 82% [IQR 68-91]), with significantly higher level of binding than T-cells and B-cells (P<0.001), and eosinophils (median 91% [IQR 83-94]). No significant differences were observed between patients with CD, UC, and healthy controls. Within the intestinal mucosa, vedolizumab mostly binds lamina propria cells, and in particular CD8+ T-cells from the terminal ileum (median 64% [IQR 28-94]). Conclusion: Differences in percentages and level of vedolizumab-binding to cell-subtypes does not explain differences in effect of vedolizumab therapy between CD and UC. These results provide baseline data for correlating vedolizumab binding capacities to clinical response in IBD patients.

IMPORTANCE OF THE RECOGNITION OF THE DENSE FINE SPECKLED PATTERN AND ANTI-DFS70 SPECIFICITY CONFIRMATION IN THE AUTOIMMUNITY LABORATORIES

R. VALENCE PEREIRA, A. MARTINEZ RODRIGUEZ, M. ESPARRAGO RODILLA, B. SACRISTAN ENCISO, S. CARRETERO CRUZ, S. GORDILLO VAZQUEZ, M. VARGAS PEREZ; SERVICIO EXTREMEÑO DE SALUD, BADAJOZ, Spain.

Background: Anti DFS70 IgG antibodies are clearly associated with some conditions including Sarcoidosis and systemic lupus erythematosus, and with some autoimmune rheumatic diseases (SARD). The recognition of the dense fine speckled pattern (DFS) in Autoimmunity Laboratories is important for the identification of these specificities. From the total number of samples tested, those compatible with DFS70 pattern were selected for further confirmatory testing for anti-DFS70 antibodies (QUANTA Flash DFS70, BIO-FIASH, Inova Diagnostics).

Methods and results: The Antinuclear Antibodies (ANA) test by IIF on Hep-2000 cells (Inmunoconcept) was requested in 15759 serum samples from 01/04/2016 to 30/06/2017. From the total number of samples tested, those compatible with DFS70 pattern were selected for further confirmatory testing for anti-DFS70 antibodies (QUANTA Flash DFS70).

Conclusions: Mono-specific anti-DFS70 antibodies are not associated with SARD.

Blood CD64+CD16+ NK cells predict corticosteron response to anti-TNFα co-medication with azathioprine in pediatric inflammatory bowel disease


Introduction: Natural killer (NK) cell subsets have recently been found to play an important role in inflammatory bowel diseases (IBD). We studied NK cell subsets before and after initiation of anti-TNFα therapy and co-medication with azathioprine (AZA) in pediatric IBD.

Materials and methods: The Study was approved by the local ethic committee (ethics protocol #347_15B) and written content was obtained from all guardians. A total of n=21 pediatric patients (Crohn’s disease, CD)/ulcerative colitis, UC) and n=9 healthy controls (HC) were recruited. Blood samples and intestinal biopsies were collected before initiation of the anti-TNFα therapy and during therapy rather than disease exacerbation. Flow Cytometry (CD3, CD8, CD16, CD56, CD62L, CCR9, β7 integrin), immunofluorescence staining (CD16, CD56), and NanoString (NanoString Technologies, Seattle, WA, USA) technique was performed. The data were compared to the clinical data derived from the medical files.

Results: Before initiation of anti-TNFα therapies, CD64+CD16+ NK cells already recognised by IIF, flow cytometry showed low numbers of peripheral CD64+CD16+ (CD, p=0.002/U/C, p=0.0001), CD65+CD16+ (CD, p=0.048/U/C, p=0.027) and y67+cells (CD, p=0.42/U/C, p=0.024) in patients with IBD compared to HC. CD-patients reaching a corticostereon <50mg/kg after initiation of anti-TNFα/AZA therapy had a significant higher relative increase of peripheral CD64+CD16+ cells beginning at 6 (1.42±0.67 vs. 0.80±0.39, p=0.0478) and 12 months (1.88±0.81 vs. 0.73±0.18, p=0.0005). Double positive CD64+CD16+ cells were reduced in pediatric IBD intestinal biopsies treated with AZA (CD, p=0.0152/U/C, p=0.0001).

Conclusions: Peripheral CD64+CD16+ NK cell subsets predict corticosteron response in pediatric IBD following anti-TNFα/azathioprine therapy.

Immunologic signaling and therapy in autoimmunity - Part 8

P.C2.08.01

Pharmacological activation of pyruvate kinase M 2 inhibits T cell activation and suppresses autoimmunity


BACKGROUND. Pyruvate kinase (PKM) catalyses the conversion of phosphoenolpyruvate to pyruvate during glycolysis, and previous studies suggested that the PKM isozyme PKM2 also has moonlighting activities different from its canonical one. However, its role in CD4+ T cell biology has never been investigated so far. The aim of this study was to study the involvement of PKM2 in T cell activation, effector functions and pathogenic activity. RESULTS. We found that PKM2 is strongly up-regulated in CD4+ T cells following CD3/CD28 activation, and can be detected in both the cytoplasm and the nucleus of resting and activated T cells, and is present in equilibrium between a monomeric (inactive) and a tetrameric (active) form. Surprisingly, pharmacological activation of PKM2 with TPEP-46, a small molecule that stabilises the tetrameric form, severely impaired T cell functions, reducing activation, proliferation and cytokine production by activating T cells. This effect was due to inhibition of hypoxia provoked factor-1 α (HIF-1α) and alteration of intracellular metabolism. TPEP-46 also induced the expression of forkhead box p3 (Foxp3) and the generation of regulatory T cells (Tregs) during T cell activation in vitro. Importantly, treatment with TPEP-46 blocked the development of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, by reducing T cell activation and inducing Treg cells in vivo. CONCLUSIONS. Our results suggest that pharmacological activation of the glycolytic enzyme PKM2 may represent a novel valuable tool for the treatment of inflammatory pathologies, and confirm that modulation of immune cell metabolic profile may have therapeutic utility in inflammation.
Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS). In healthy donors (HD), autoreactive T cells are suppressed by regulatory T cells (Tregs). In relapsing-remitting (RR)MS, patients, blood-circulating Tregs are defective. Little is known about their presence in and migration to the CNS. Here, the migratory phenotype and capacity of Tregs are investigated. Using an in vitro model of the human blood brain barrier (BBB), we found that Tregs have a migration frequency of 8% across an infiltrated BBB. Flow cytometry analysis of paired blood and cerebrospinal fluid (CSF) samples of untreated RRMS patients shows comparable percentages of Tregs (CSF: 6.8%; blood: 8.4%), indicating their capacity of migrating to the CNS. To understand the mechanism of migration, Tregs and BBB endothelial cells (EC) were phenotyped. The percentage of CD11a+ Tregs is significantly decreased in blood of untreated RRMS patients (43.3%) compared to HD (51.2%, p<0.019). In contrast, the CD11a+ ligand, ICAM-1, is significantly increased on infiltrated BBB EC. With regard to chemokine receptors, we found that the percentage of CCR5+ Tregs is significantly increased in blood of RRMS patients (39.6%) compared to HD (26.5%, p<0.05). Furthermore, CD45RO+CCR8+ and CCR9+ Tregs are enriched in the CSF of untreated RRMS patients. The CCR5 ligand, CXCL10, is significantly upregulated in infiltrated EC. These results suggest that Tregs use a specific set of adhesion molecules and chemokine receptors to migrate to the CNS of MS patients. Further results of ongoing analyses will be presented at the meeting.

P.C2.08.02
Regulatory T cells have a distinct migratory phenotype in relapsing-remitting multiple sclerosis patients
P. Boeten, N. Hellings, B. Broux;
Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium.

P.C2.08.03
Infection risk assessment during immunosuppressant treatment using computer simulations
K. Beuke, M. Rebherg, A. Dietrich, T. Klubanje, D. Seren Alp, N. Biesemann, C. Asbrand;
Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany.

P.C2.08.04
GLUTATHIONE CONTROLS ROS TO PRIME T CELLS FOR INFLAMMATION
1The Campbell Family Institute for Breast Cancer Research, Toronto, Canada, 2LH, Eisch-scher-Alzette, Luxembourg, 3Tokyo Medical and Dental University, Tokyo, Japan, 4University of Braunschweig, Braunschweig, Germany, 5University of Marburg, Marburg, Germany, Ensurch-scher-Alzette, Luxembourg, 6Australian National University, Canberra, Australia, 7School of Public Health, New Haven, United States, 8University of Marburg, Marburg, Germany, 9Odense University Hospitals, Odense, Denmark, 10University of Dusseldorf, Dusseldorf, Germany, 11Harvard Medical School, Boston, United States.

P.C2.08.05
The diagnostic value of the antiaging protein, Klotho in early and late stages of multiple sclerosis
M. Emami Aleagha, A. Allameh, M. Harricharian, S. Rosami, S. Lavasani, M. Javan, M. Pahlevan Kokhali;
1Department of Research of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 2Islamic Republic of, 3Iranian Center of Neurological Research, Neuroscience Institute, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran, 4Islamic Republic of, 5Department of Biology, Sileventag 35, Building C, SE-223 62, Lund, Sweden, 6Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 7Islamic Republic of, 8Department of Clinical Neuroscience, Center for Molecular Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

P.C2.08.06
The regulatory role of the antiaging protein, Klotho in experimental autoimmune encephalomyelitis
Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany.

P.C2.08.07
Intravenous MMSC treatment ameliorates inflammation in experimental autoimmune encephalomyelitis
C. L. Freitas, C. M. Polonio, W. N. Brändi, N. G. Zoniugu, L. G. Oliveira, C. Cerezas, I. P. Evangelista, S. Halper, M. G. Nisenbaum, M. Maluf, P. Perin, J. S. Peron;
1Institute of Biomedical Science, São Paulo, Brazil, 2Célula Mater, São Paulo, Brazil, 3Célula Mater, São Paulo, Brazil, 4Célula Mater, São Paulo, Brazil.

INTRODUCTION: Multiple sclerosis (MS) is a neurodegenerative autoimmune disease that leads to demyelination of neuronal axons. Filopodial tubes are a source of mesenchymal stromal cells in humans (hMSCs) and mice (mMSCs), which are undifferentiated multipotent cells that can橇 role in autoimmunity diseases due its immunomodulatory properties. Since the management of symptoms remains unclear, we propose to evaluate the immunomodulatory effect of hMSCs and mMSCs using the murine model of MS, the Experimental Autoimmune Encephalomyelitis (EAE).

METHODS: C57BL/6 mice were immunized with MOG35-55. We observed a significant increase in the expression of Klotho gene in spinal cord tissue. Conclusion: Klotho has a potential candidate for diagnosis, monitoring and also treatment of MS.
P.C2.08.07
Combinatorial associative study of IL10, IL18 and TNF-α gene polymorphisms in relapsing-remitting multiple sclerosis
B. Grigorov1, A. Trenova2, L. Miteva1, S. Staniilov1;
1Medical Faculty, Trakia University, Stara Zagora, Bulgaria, 2Faculty of Medicine, Medical University, Plovdiv, Bulgaria.
Introduction: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system leading to neurological dysfunctions. The aim of the present study was to investigate the association between the promoter polymorphisms in IL10 (rs1800896), IL18 (rs1946518), TNF-α (rs1800629) and genetic predisposition to relapsing-remitting multiple sclerosis (RRMS) in Bulgarian patients.
Materials and Methods: Our case-control study includes 159 RRMS patients with DMT therapy (114 women and 45 men, 40.08±18.48 years) and 125 women and 44 men (39.27±18.85 years) age-sex-matched healthy volunteers. All included subjects were genotyped by ARMS-PCR and RFLP-PCR methods.
Results: Our results revealed no significant differences of studied polymorphisms between cases and controls: IL10 (χ²=0.493; p=0.782); IL18 (χ²=4.073; p=0.130) and TNF-α (χ²=3.153; p=0.207). However, we must note that homozygous for variant allele-A in IL18 polymorphism was not detected in the patient’s group in contrast to control. Also, for rs1800629 in TNF-α, it was calculated OR=1.505 for the heterozygous genotype. When we combined both IL10 and IL18 polymorphisms, we detected the lower frequency of AG+GG/CC genotypes compared to the wild genotypes AA/CC (OR=0.481, 95% CI=0.199-1.157, p=0.073). In the same line we were the results when was added the wild genotype of TNF-α polymorphism (GG) to the combination of IL18 and IL18 genotypes (GG/AA/CC vs GG/AG/GG/CC, OR=0.446, 95% CI=0.165-1.189, p=0.073).
Conclusion: Our present results indicate that the carrying of variant allele-G in IL10 (AG+GG genotypes) in combination with both wild genotypes of IL18, and of TNF-α polymorphisms, might influence the risk of RRMS susceptibility in Bulgarian patients.

P.C2.08.08
Association study of HLA class I antigens with central nervous system inflammatory diseases
S. Mejdoub1, A. Charfi2, A. Kamoun1, M. Mahfoudi1, S. Feki1, M. Dammak1, H. Hachicha3, L. Maalej1, I. Kamoun1, F. Hakim1, L. Gaddour2, B. Mallek1, C. Mhiri1, H. Masmoudi1, H. Makni1;
1Immunology Laboratory, Habib Bourguiba Hospital, Sfax, Tunisia, 2Histocompatibility Laboratory, Hedi Chaker Hospital, Sfax, Tunisia, 3Neurology Department, Habib Bourguiba Hospital, Sfax, Tunisia.
Central nervous system (CNS) inflammatory diseases include multiple sclerosis (MS) but also neurological manifestations of systemic diseases such as Behçet disease. As the association of HLA-B51 antigen with Behçet disease is well established, our aim was to evaluate the frequency of this antigen in patients with CNS inflammatory disease suspicion. Patients followed in neurology department for CNS inflammatory disease suspicion (June 2014-May 2016) for whom HLA class I typing was performed (microlymphocytoxicity complement dependent technique) were included. Our control population included 123 unrelated healthy subjects. Statistics were studied according to Microsoft Excel.
Thirty patients (17 men and 23 women) were included. The established diagnosis was MS in 27 cases, clinically isolated syndromes (CIS) in 8 cases, Behçet disease in 2 cases and CNS vascular disease in 3 cases. Among these patients, 10 (7 MS and 3 CIS) expressed HLA-B51 antigen (25% VS 9,76% in controls, p=0,01; OR=3,08). Comparing MS patients with controls, HLA-A10 antigen was significantly associated with the disease (25,93% VS 9,76%, p=0,04; OR=3,24). All patients expressing HLA-A10 antigen belonged to MS group. Our study, despite concerning a small number, showed an association of HLA-B51 antigen with CNS inflammatory diseases particularly MS. This can probably be explained by linkage disequilibrium between this antigen and HLA-DR15, reported as a susceptibility marker for MS. Association of HLA-A10 with this disease probably the involvement of another susceptibility region telomeric to HLA-B locus. A bigger patients population size is required to confirm our preliminary results.

P.C2.08.09
Regulatory T cell-driven suppression of experimental autoimmune encephalomyelitis during pregnancy
N. Heckmann1, J. B.Engler1, C. Ramien1, S. M. Gold1, M. A. Friese1;
1Institut für Neuroimmunologie und Multiple Sklerose, Zentrum für Molekulare Neurobiologie Hamburg, Universitätshospital Hamburg-Eppendorf, Hamburg, Germany, 2Charité Universitätsmedizin, Berlin, Germany.
Pregnancy reduces disease activity of several autoimmune inflammatory diseases including multiple sclerosis (MS), where the relapse rate is reduced by approx. 80% in the third trimester. This protection is mimicked in experimental autoimmune encephalomyelitis (EAE), the animal model of MS. Regulatory T cells (Treg) play a pivotal role in controlling both feto-reactive T cells during pregnancy and auto-reactive T cells in autoimmune diseases, however, it remains unknown how Treg exert their increased suppressive capacity during pregnancy. We have recently reported that Treg increase during pregnancy and that the protective effect on EAE is dependent on regulation via the glucocorticoid receptor in T cells. In the present study, we surveyed energy metabolism, phenotype and T cell receptor (TCR) repertoire of Treg during pregnancy to determine the relative contributions of these factors to pregnancy-induced Treg activity and protection from autoimmunity. We detected no changes in energy metabolism in Treg during pregnancy in mice. Next, we probed the TCR repertoire of Treg and Tcon at different pregnancy stages and under EAE conditions and are currently monitoring the relative distribution of suppressive clones. To understand Treg suppressive capacity and differentiation changes during pregnancy, we extensively phenotyped and functionally analysed Treg from pregnant and non-pregnant EAE mice. Together, our findings give a comprehensive understanding of the pregnancy-induced changes in Treg and how these changes contribute to an amelioration of EAE disease activity. This work is funded by the DFG KFO 296.

P.C2.08.10
Multiple Sclerosis associated cytotoxic CD4+ T cells escape regulatory T cell mediated suppression
C. Hoeks1, M. Vanheusden, L. Peeters, P. Snitsenis, B. Broux, N. Hellinga1;
1Hasselt University, Biomedical Research Institute and Transnationale Universiteit Limburg, Diepenbeek, Belgium.
A terminally differentiated subset of CD4+ T lymphocytes, characterized by loss of the costimulatory molecule CD28 and gain of cytotoxic activity, arises during aging and chronic inflammation and age-inappropriate expansion of these cells has been found in autoimmune diseases like multiple sclerosis (MS). Our group has recently published that CD4+ cytotoxic T lymphocytes (CTL) contribute to the pathology of MS. We showed that expansion of CD4+ CTL exacerbates experimental autoimmune encephalomyelitis. In addition, we found that presence of peripheral CD4+ CTL is directly linked to MS disease severity and that this holds value as a novel prognostic marker in MS. However, the mechanism behind these findings remains unclear. Here we show that CD4+CD28null T cells are phenotypically distinct from CD4+CD28+ T cells, and that CD4+CD28null T cells evade Treg-mediated suppression in vitro. CD4+CD28null T cells display enhanced levels of pro-inflammatory molecules such as granzyme B, IFN-gamma, IL-1beta, IL-6, IL-22, and GM-CSF, but decreased levels of IL-10R and GITR. Tregs upregulate IL-10, granzyme B, CTLA-4, and IFN-gamma when exposed to the secretome of CD4+CD28null T cells. An in vitro co-culture system has been optimized to further analyze how CD4+ T cells contribute to the pathology of MS.

P.C2.08.11
B cell subpopulations in the pathogenesis of multiple sclerosis
Danish Multiple Sclerosis Center, Copenhagen Ø, Denmark.
B cells are important contributors to the pathogenesis of multiple sclerosis (MS), where they regulate the inflammatory immune response and participate in development of lesions in the CNS. Dimethyl fumarate (DMF) is used to treat patients with relapsing-remitting MS (RRMS); however, its impact on B cell subpopulations remains uncertain. In this study we therefore investigated the phenotype of B cell subpopulations in 18 untreated patients with RRMS, 17 healthy controls (HC) and 21 patients treated with DMF for more than 12 months. Using flow cytometry, B cell subpopulations were defined according to their expression of CD27 and CD38. This showed that CD27-CD38- B cells were increased in the blood of untreated patients compared to HC. We also found that CD27-CD38- B cells migrated to the CSF of untreated patients; proposing an association with MS pathogenesis. When patients were treated with DMF we found a treatment-induced reduction in the frequency of CD27-CD38- B cells and CD27+ memory B cells. We also found that the observed reduction in antigen-experienced B cells in DMF-treated patients likely was due to a reduced frequency of follicular helper T(FH) cells and an increased frequency of follicular regulatory T (TFR) cells. Using the in vitro T1 assay, we observed that the frequency of T1a, T1d, T1f, T1g and T1h producing B cells increased in the frequency of TGF-β producing cells. These data demonstrate an anti-inflammatory role of DMF on the B cell compartment.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands.

P.C2.08.12
Targeting Plasma Cells with Proteasome Inhibitors for Treatment of Myasthenia Gravis
M. Mané Damas, M. Losen, M. Pilar; Maastricht University, Maastricht, Netherlands.

Autoantibodies against the muscle AChR are mainly produced by both short- and long-lived plasma cells, which are resistant to standard immunosuppressive drugs (e.g. glucocorticoids). A novel therapy to eliminate plasma cells is the proteasome inhibitor bortezomib, which is used to treat patients with multiple myeloma (MM, a plasma cell malignancy). Previously, we demonstrated that bortezomib also reduced autoantibody titters in an animal model of MG (Gomez, A. M. J. Immunol. 2011). The thymus of MG patients often contains plasma cells which are established in germinal centers after irradiation (which depletes B and T lymphocytes). We studied the in vitro effects of bortezomib in cultured thymus cells from MG patients undergoing therapeutic thymectomy. Treatment with a single dose of bortezomib eliminated plasma cells and thereby blocked the production of IgG, including pathogenic autoantibodies. Ultrastructural signs of apoptosis were detected in plasma cells as early as 8 h after addition of bortezomib; at 24 h, no plasma cells could be detected (Gomez, A. M. J. Immunol. 2014). Finally, we are currently testing in vitro and in vivo second-generation proteasome inhibitors efficient in eliminating autoreactive plasma cells with special focus in investigating their side effects such as peripheral neuropathy.

P.C2.08.13
Anti-NF155 chronic inflammatory demyelinating polyradiculoneuropathy strongly associates to HLA-DRB15

OBJECTIVE: To study the human leukocyte antigen (HLA) class II allele frequencies in chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) associated with anti-neurofascin 155 (NF155) antibodies. METHODS: Three dozen anti-NF155+ and 35 seronegative CIDP patients were included in a case-control study. The frequencies of the DRB1 HLA alleles were analyzed in all patients while DR frequencies were only studied in patients sharing the DRB1*15 allele. In silico HLA-peptide binding and NF155 antigenicity predictions were performed to analyze overlap between presented antigens and peptide regions. RESULTS: DRB1*15 alleles (DRB1*15:01 and DRB1*15:02) were present in 10 out of 13 anti-NF155+CIDP patients and in only 5 out of 35 seronegative CIDP patients (76.9% vs 14.2%; OR=20, CI=4.03 to 99.13). DRB1*15 alleles appeared also in significantly higher proportions in anti-NF155+CIDP than in normal population (76.9% vs 16.5%; OR=16.9, CI=4.43 to 57.30). Seven anti-NF155+ CIDP patients (52%) and 5 seronegative CIDP patients had the DRB1*15:01 allele (OR=7.7, p<0.0009), while 3 anti-NF155+ CIDP patients and none of the seronegative CIDP patients had the DRB1*15:02 allele (OR=23.6, p=0.016). In silico analysis of the NF155 peptides binding to DRB1*15 alleles showed significant overlap in the peptides presented by the peptides presented by the 15:01 and 15:02 alleles, suggesting functional homology. CONCLUSION: DRB1*15 alleles associate strongly to anti-NF155 antibodies CIDP and provide additional evidence to support that these patients constitute a differentiated CIDP subset.

P.C2.08.14
Teriflunomide induces in multiple sclerosis a tolerogenic phenotype in innate immune cells resulting in a reduction in terminally differentiated effector lymphocytes.

Introduction: Teriflunomide is a disease modifying treatment approved for multiple sclerosis (MS). This molecule inhibits reversibly dihydro-orotate dehydrogenase, a mitochondrial enzyme involved in de novo pyrimidine biosynthesis, and down-regulates proliferation of activated lymphocytes. However, there are few data on the impact of this drug on the lymphocyte subsets. Methods: We studied 50 MS patients treated with teriflunomide. Results: Methods: 55 patients with relapsing-remitting MS who initiated teriflunomide treatment were included in the study. We studied peripheral blood mononuclear cells obtained before and six months after treatment initiation and exploratory effect on lymphocyte subsets. Results: When explored T and B cell subsets, we observed a decrease in the percentages of terminally differentiated CD4+ T cells (p=0.001) and plasmablasts (p<0.0001) after 6 months of treatment. In addition, when explored T cell subsets, we observed an increase in the percentage of terminally differentiated CD8+ T cells (p=0.016). CONCLUSIONS: Teriflunomide induces a specific reduction in effector T and B cells that have shown to play a role in MS course and an increase in regulatory cells. This drug induces the expression of PD-L2, a molecule involved in toleration to autoantigens, which can contribute to inhibit the abnormal immune response taking place in MS.

P.C2.08.15
Biomarkers for early prediction of dimethyl fumarate associated lymphopenia in multiple sclerosis
S. Medina, S. Sainz de la Maza, N. Villarrubia, E. Rodriguez-Martín, L. Costa-Fressard, A. Tejeda-Velarde, E. Roldán, J. C. Álvarez-Cermeño, E. Roldán, L. Villar; Ramón y Cajal Hospital, Madrid, Spain, 3Clínico San Carlos Hospital, Madrid, Spain, 3Quirónsalud Madrid Hospital, Madrid, Spain.

Introduction: Dimethyl fumarate (DMF) is a first line treatment for relapsing remitting multiple sclerosis (MS). Lymphopenia is a major concern in MS patients treated with DMF as it increases the risk of serious infectious side effects. Predicting which patients have an increased risk of developing lymphopenia could have important implications for personalized therapy in these patients. The main goal was to identify factors predicting lymphopenia in DMF-treated patients. Methods: Prospective longitudinal study including 106 patients initiating DMF treatment. They were followed for a mean time of 23.58 months, and monitored every three months. Blood lymphocyte subsets were studied in a representative group of 64 patients by flow cytometry at baseline and 6 months after. Results: Mean absolute lymphocyte counts (ALCs) decreased by 29% during the first year of DMF-treatment. Patients developing lymphopenia showed a faster decline within the three first months. A reduction of ALCs higher than 36% at this time, accurately predicted subsequent lymphopenia (OR=7.35, 95% CI 1.0-7.9, p=0.0001). We classified patients in two groups according to the appearance of lymphopenia. Both showed a significant decrease in effector memory T cells, total and terminally differentiated CD8+ T cells and memory B cells upon DMF therapy. In addition, non-lymphopenic patients experienced a selective increase in naïve CD4+ T cells, not experienced by those presenting lymphopenia. CONCLUSIONS: A decline in ALCs below 36% after 3 months of DMF-treatment identifies patients with low probability of developing lymphopenia. This decrease may be associated with retardation in the production of naive CD4+ T cells.

P.C2.08.16
The inhibition of dopamine receptor D3 signalling in CD4+ T-cells exerts a therapeutic effect attenuating Parkinson's disease development in a mouse model
R. Pacheco1, D. Elgueta1, F. Contreras, C. Pradov, M. A. Abellanas, M. S. Aymérich, R. Franco1
1Fundación Ciencia & Vida, Santiago, Chile, 2Department of Biologics, Faculty of Biologies, Universidad Andres Bello, Santiago, Chile, 3Division of Neuroscience, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain, 4Department of Biochemistry and Molecular Biomedicine, University of Barcelona, Barcelona, Spain.

Neuroinflammation constitutes a fundamental process involved in Parkinson's disease (PD). Microglial cells play a central role in the outcome of neuroinflammation and consequent neurodegeneration of dopaminergic neurons in the substantia nigra.Current evidence indicates that CD4+ T-cells infiltrate the brain in PD, where they play a critical role determining the functional phenotype of microglia, thus regulating the progression of the disease. Recently, we demonstrated that mice bearing dopamine receptor D3 (DRD3)-deficient CD4+ T cells are completely refractory to neuroinflammation and consequent neurodegeneration induced by the intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In this study we evaluated the therapeutic potential of targeting DRD3 in CD4+ T-cells by inducing the pharmacologic or the transcriptional inhibition of DRD3-signalling in a mouse model of PD induced by the chronic administration of MPTP and probelecid (MPTPP). We also analysed whether DRD3-signalling was altered in the immune cells of PD patients. The results show that the transference of CD4+ T-cells transduced ex vivo with retroviral particles coding for an shRNA for DRD3 or treated ex vivo with the DRD3 antagonist PF0037730 into MPTP-mice resulted in a significant reduction of motor impairment and neuroinflammation. In vitro analyses showed that the frequency of peripheral blood Th1 cells, a phenotype that is promoted by DRD3-signalling, was significantly increased in PD patients. Nevertheless, DRD3 expression was completely reduced in CD4+ T-cells obtained from PD patients. Our findings indicate that attenuating DRD3-signalling in CD4+ T-cells exerts a therapeutic effect in parkinsonian animals dampening motor impairments and modifying the pro-inflammatory phenotype of glial cells.
POSTER PRESENTATIONS

P.C2.08.17 A fusion protein of IL4 and IL10 to resolve inflammatory pain
J. Prado1, R. H. Westerink1, J. Popov-Celekovic1, C. Steen-Louws1, A. Pandit1, W. Worp van de1, K. A. Reedquist1, L. Koenderman1, C. E. Hack2, N. Eijkelkamp1
1UMC Utrecht, Utrecht, Netherlands, 2Institute for Risk Assessment Sciences, Utrecht, Netherlands.

Chronic pain is difficult to treat and new therapeutic approaches to treat it are highly needed. Anti-inflammatory cytokines have potential to resolve chronic pain, but their most optimal in concert of each other. We developed a novel approach to optimize the potential of anti-inflammatory cytokines to resolve persistent pain by fusing IL4 and IL10 into one molecule. Intrathecal administration of IL4-10 fusion protein (FP) completely resolves inflammatory pain in multiple animal models, in a superior fashion than the combination of individual cytokines. In addition, in vitro, IL4-10 FP inhibited TNFs and PGE2, induced neuronal nitric oxide synthesis and caspase-induced calcium fluxes. Importantly, equimolar concentrations of IL4-10 FP more effectively inhibited neural sensitization than the combination of individual cytokines. Mechanistically, we show that IL4-10 FP, in contrast to the combination of IL4 and IL10, clusters IL4R and IL10R in sensory neurons as a potential mechanism for the increased effectiveness. In vivo, conditional knockdown of IL10R in Nao1.5+ microglia and IL4R in sensory neurons using intrathecal antisen anti-oligodeoxynucleotides completely ablated the IL4-10-mediated inhibition of inflammatory pain. Knockdown of IL4R or IL10R only was sufficient to prevent the superior effect of IL4-10-mediated pain inhibition. Finally, intrathecal administration of IL4-10 FP induced a completely different kinome and transcriptome profile in the DRG compared to IL4+IL10 treatment. These data underscore that anti-inflammatory cytokines can be used to target the sensory system to fully resolve persistent pain. Moreover, they show that IL4-10 FP achieved unaltered biological effects that are more than the mere sum of the two cytokines.

P.C2.09.19 Assessment of anti-DPS70 antibodies in the diagnostic approach of Systemic Autoimmune Rheumatic Diseases
A. Tsirigotten1, A. Giannakou1, K. Soufkerou1, M. Bantadaki1, E. Synodinou1, A. Markantontanou2, E. Pipi1, A. Pavlou2
1Immunology-Histocompatibility Dept. “Evaggelismos” General Hospital, Athens, Greece, 2Immunology-Histocompatibility Dept. “Papageorgiou” General Hospital, Thessaloniki, Greece.

Antinuclear antibodies (ANA) are a serological hallmark of systemic autoimmune rheumatic diseases (SARD). However, positive ANA with a dense fine speckled (DFS) pattern (Hep-2), have been reported in other clinical entities. Interestingly, the anti-DPS70 antibodies are not significantly associated with SARD.

Aim: The aim of the study was the investigation whether the use of a protocol that pre-absorbs anti-DPS70 antibodies from the sera, increases the diagnostic efficiency of Hep-2 ANA testing for SARD.

Subjects and methods
Positive ANA-IF consecutive sera with DFS pattern from 120 patients with SARD and 170 patients with other clinical entities (non-SARD) referred to our Departments for ANA, DNA and extractable nuclear antigen (ENA) antibodies testing, were retrospectively tested using the Nova NovaLite Hep-2 Select Kit for ANA-IF.

Results: The positive ANA-IF after anti-DPS70 pre-absorption was 94.1% (113/120) in SARD patients, while in non-SARD patients the positivity dropped to 44.7% (79/170).

Conclusion: Based on our results we recommend that the anti-DPS70 pre-absorption protocol on ANA-Hep-2 specificity for SARD, but more studies are needed to address whether it decreases the respective sensitivity.

P.C2.08.20 Interaction between the HLA-Shared Epitope (SE) and smoking in anti-CCP positive Greek patients with Rheumatoid Arthritis
K. Tarasoiou1, E. Moile1, V. Kitisou1, D. Kouniaki1, V. Athanasioadi1, K. Soufkerou1, E. Synodinou1, A. Petras2, C. Sfontouris2, A. Tsirigotten1
1Immunology-Histocompatibility Dept. “Evaggelismos” General Hospital, Athens, Greece, 2Rheumatology Dept. “Evaggelismos” General Hospital, Athens, Greece.

Genetic and environmental factors involve in etiopathogenesis of Rheumatoid Arthritis (RA). The aim of the study was the assessment of association of HLA-DRB1*1-SE in the Greek population in autoimmunity and therapy in autoimmunity - Part 9.

Material and Methods: Chronic C57BL/6 EAE and relapsing-remitting SJL/J EAE were induced and treated with ACDT. The effect of ACDT on pathogenic T cell infiltration, microglia activation, neurotoxic A1 astrocytes, blood-brain barrier (BBB) integrity in the CNS of EAE was assessed.

Results: ACDT, administered post immunization, delayed disease onset and reduced disease severity in chronic C57BL/6 EAE, and ACDT, administered during disease remission, reduced inflammation and promoted remission in chronic EAE.

Conclusions: In the current study we evaluated the therapeutic effect of 5-Amino-3-thioxo-3H-(1,2)dithiole-4-carboxylic acid ethyl ester (ACDT), a substituted derivative of D3T, in EAE. Structurally-simplest of the sulfur-containing dithiolethiones, exerted a promising therapeutic effect in EAE. In the current study we evaluated the therapeutic effect of 5-Amino-3-thioxo-3H-(1,2)dithiole-4-carboxylic acid ethyl ester (ACDT), a substituted derivative of D3T, in EAE.

P.C2.08.21 Dithiolethione ACDT suppresses autoimmunity and ameliorates disease severity in experimental autoimmune encephalomyelitis
P. Kuo1, D. A. Brown2, B. A. Sovcikfield3, H. C. Parasou1, I. Yli1, J. Yen1
1Indiana University School of Medicine, Fort Wayne, United States, 2Manchester University College of Pharmacy, Fort Wayne, United States.

Introduction: Multiple sclerosis (MS) is a autoimmune disease characterized by the central nervous system (CNS) inflammation of myelin-specific pathogenic T cells followed by brain inflammation and demyelination. In the CNS, these pathogenic T cells, including Th1 and Th17, leading to detrimental effect of demyelination. We previously reported that 3H-1,2-dithiole-3-thione (D3T), the structure-simplyest of the sulfur-containing containing biosynthesis, expressed a promising therapeutic effect in MS. Interestingly, the anti-DFS70 antibodies are not significantly associated with SARD.

Methods and Materials: Chronic EAE in mice and relapsing-remitting EAE in mice were induced and treated with ACAT. The effect of ACAT on pathogenic T cell activation, microglia activation, neurotoxic A1 astrocytes, brain-blood barrier (BBB) integrity in the CNS of EAE was assessed.

Results: ACAT administered post immunization, delayed disease onset and reduced disease severity in chronic EAE and EAE, administered during disease remission, suppressed disease relapse in relapsing-remitting EAE. Further analysis of the cellular and molecular mechanisms underlying the protective effects of ACAT in EAE revealed that ACAT inhibited pathogenic T cell infiltration, suppressed microglia activation, repressed neurotoxic A1 astrocyte generation, lesioned BBB disruption, and diminished MKM9/9 production in the CNS of EAE.

Conclusions: We demonstrate that ACAT suppresses autoimmunity and ameliorates disease severity in EAE through multiple cellular mechanisms. Our findings suggest the potential of developing ACAT as a novel therapeutic agent for the treatment of MS/EAE.

P.C2.09.1 Immune signaling and therapy in autoimmunity - Part 9

A. Alkakhi1, M. Pahlavan Kakhi2, M. Behmanesh1
1Department of Molecular Genetic, Tarbiat Modares University, Tehran, Iran, Islamic Republic of; 2Department of Clinical Neuroscience, Center for Molecular Medicine, Karolinska Institutet, Karolinska Hospital, Stockholm, Sweden.

Introduction: Coronary artery disease (CAD) can be classified as an inflammatory disease, which affected by type 2 diabetes mellitus (T2DM). Elevated levels of many inflammatory molecules were found in the serum of patients with CAD. SOCS3 molecule is a cytokine-induced inhibitor of STAT3. STAT3 molecules are involved in the regulation of cardiovascular diseases. The expression levels of STAT3 and its important regulatory genes Inc-DC and SOCS1, in patients with CAD and T2DM. Methods: Blood samples were obtained from 37 CAD+ and 36 CAD− patients. These patients were enrolled in this study based on angiography findings and categorized based on T2DM status. The expression levels of STAT3, Inc-DC, and SOCS1 genes were examined with Real-time PCR method. Results: A significant increase was observed in the expression of STAT3 and Inc-DC genes but not SOCS1 in CAD+ versus CAD− patients. These results elucidated partially in some groups categorized based on T2DM and CAD status. However, the severity of CAD had no effect on expressions of these genes. Moreover, we found some significant correlations between expressions of Inc-DC with SOCS1 and STAT3, which confirmed by in silico analysis. Conclusion: Our results shed further light on the inflammatory aspects of CAD and T2DM with emphasis on JAK/STAT pathway and the regulatory role of long non-coding RNAs in the physiopathology of these diseases.
Regulatory T cells (Treg) are of paramount importance for restraining excessive immune responses and their manipulation holds enormous therapeutic potential. Our recent results using a congenic rat model suggested that the integrity of Vav1/Themis1 T-cell receptor signaling hub plays a crucial role in Treg lineage stability and suppressive function. Indeed, Themis1 deficiency in BN, but not in LEW rats, led to the development of inflammatory bowel disease (IBD), linked to high levels of IL-17 and IFNγ production by Treg, and alterations in their suppressive function. Genetic studies revealed that this phenotype depended on a 117 Kb genomic locus, containing the REL3W polymorphism on Vav1 that impacted its expression and functions. To test the importance of the Vav1/Themis1 TCR signaling hub in Treg function, we generated Themis1−/− mice expressing conditionally Themis1 in thymocytes, but not in peripheral T cells. In contrast to regular germline Themis1 knockout mice, these mice were not lymphopenic and exhibited normal proportions of CD4+ T cells in the thymus and in peripheral lymphoid organs. Next, Themis1−/− mice were crossed with Vav1−/− mice to assess the impact of these combined mutations on Treg suppressive function and the potential role of these mutations in vivo approaches. Together with in vivo analyses of IBD or melanoma models, we showed that suppressive activity of Treg was impaired in Themis1-deficient mice harboring the mutated Vav1. Functional studies showed the implication of SHP1 in these functional defects. Together, these data showed that Themis1 and Vav1 cooperate in the same signalosome to regulate the suppressive function of regulatory T cells.

P.C.2.09.04
Glucocorticoid hormone treatment enhances the cytokine production of regulatory T cells by upregulation of Foxp3 expression

T. Berkí, L. Prenek, E. Ugör, R. Pap, F. Boldszás, P. Németh
Department of Immunology and Biotechnology, University of Pécs, Pécs, Hungary.

Objective: Despite the fact that glucocorticoids (GC) are important therapeutic tools, their effects on regulatory T cells (Treg) are not well defined. The aim of our work was to investigate how GCs influence in vivo the Thymic (Treg) and peripheral Treg (pTreg) differentiation, survival and cytokine production.

Methods: Tregs were detected with flow cytometry in lymphatic organs of 4-6 weeks old BALB/c mice after repeated (2-4 days), high-dose GC treatment using CD4/CD25 cell surface and Foxp3/IL-10/GFPI/glucocorticoid receptor (GR) intracellular staining. Cytokine, Foxp3, and GR mRNA levels of sorted CD4+/CD25+ T cells were analyzed using RT-PCR.

Results: GC treatment resulted in increased relative Treg frequency in the lymph nodes, with unchanged absolute cell count. The pTreg ratio and absolute cell count decreased in secondary lymphatic organs. Elevated intracellular IL-10+ and TGFB+ expressing pTreg and pTreg ratios were measured in GC-treated animals, accompanied with elevated Foxp3 mRNA expression. GC treatment increased TGFB and IL-35 mRNA in spleen and elevated IL-10 mRNA in thymic Tregs. GC induced nuclear localization of GR in both Tregs and pTregs, which correlated with high colocalization (>40%) with Foxp3. These data suggest an interaction of these two transcription factors with further increase due to GC treatment in pTregs.

Conclusion: Our data show selective upregulation of Tregs and elevated production of immunosuppressive cytokines by Treg cells after GC treatment, which may contribute to the immunosuppressive effects of GCs.

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P.C.2.09.05
Expression of PD-1 and PD-L1 markers on T-regulatory cells during cytotoxic- and anti-CD3-induced proliferation in norm and rheumatoid arteritis

E. A. Blinova, E. A. Patchkina, O. V. Shevryev, L. V. Grishina, A. E. Sizikov, V. A. Kozlov
Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

Rheumatoid arteritis (RA) characterized by disruption of tolerance and Treg dysfunction. The aim of our study was to evaluate expression of functional molecules on Treg under cytokine-induced proliferation in norm and RA.

The study included 6 patients with RA and 7 healthy donors (average 60±4,4 and 60±4,5 years respectively). After isolation PBMCs were cultivated with or without IL-7 (50ng/ml), IL-15 (50ng/ml) and simultaneously IL-7+IL-15, anti-CD3 antibodies (1mkg/ml) and IL-2 (100ME/ml) during 7days. Analysis of PD-1 and PD-L1 expression on CD4+ T cells was performed by flow cytometry (CantoII BD).

Groups of donors and patients didn’t differ in number of PD-1 and PD-L1 expression in peripheral blood. Culturing of PBMCs without stimulation led to a decreasing of Treg in both groups, and conversely - to an increasing of density of CD25 and CD127, perhaps, due to lack of growth factors. Activation by homostatic factors promotes the increasing of PD-L1 expression on Treg, on the lower level than of IL-7 and IL-15 stimulation. In RA, after stimulation with IL-7 it was demonstrated more PD-1-positive and less PD-L1-positive Tregs than in norm. Under IL-15 and IL-15+IL-2 stimulation, patients had less PD-1+ Tregs compared to donors. It was an increase in number of PD-L1+ Tregs after IL-25+IL-2 stimulation in RA compared to norm.

In RA changes in expression of PD-1 and PD-L1 on Tregs under the high doses of IL-7 and IL-15 can influence on capacity of Tregs to control proliferation of auto-reactive T-cell clones. The reported study was funded by RFBR and Novosibirsk region, the research project No.17-44-540167.

P.C.2.09.06
Identification of VIMP as a novel anti-inflammatory gene of CD4+ effector T cells by a correlation network-guided approach

C. M. Vojnović, 1,2 N. Ženić, 1,2 E. Danilecviciute, 1,2 M. Ollert 1,2, R. Bolling, 1,2 F. Héry 1,2
1Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg, 2University of Luxembourg, Esch-sur-Alzette, Luxembourg, 1Odense Research Center for Anaphylaxis (ORCA), Department of Dermatology and Allergy Center, University of Southern Denmark, Odense, Denmark, 2Luxembourg Center for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg.

Although many key players of the CD4+ T cell inflammatory response have already been identified, many nodes of the underlying network are still missing for a deep understanding of the molecular mechanisms. We previously established a correlation network-guided strategy to identify novel key hub genes and extended the strategy to identify novel genes regulating the inflammatory response of CD4+ effector T cells (Teffs), based on high-resolution time-series data of human Teffs in the first 6 hours following T cell receptor stimulation. We identified VIMP (VCP-interacting protein, also known as SELS, SELENOS), one of the 25 genes encoding the 21st amino acid selenocysteine in humans, as a novel gene involved in Teff function. VIMP is known to be an important component of the endoplasmic reticulum (ER)-associated degradation (ERAD) complex and has functions in cell survival by regulating ER stress. Furthermore, VIMP has been shown to have an anti-inflammatory function in macrophages.

Knocking-down VIMP in Teffs significantly enhanced their proliferation and the expression of different cytokines. By using a computational approach, we predicted the transcription regulatory- and signalling transduction network through which VIMP regulates cytokine expression, based on the dynamic correlation network of Teffs and on transcriptome analysis, and experimentally validated the prediction in a finer scale. Altogether we demonstrate that VIMP inhibits the inflammatory response via both the E2F5 transcription factor and the phosphorylation of NFATC2 through which VIMP regulates the inflammatory response in CD4+ effector T cells.

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POSTER PRESENTATIONS

P.C2.09.08

IL-17A/F contributes to the pathogenesis of experimental Epidermolysis Bullosa Acquisita

F. Deng1, I. Pizzi1, X. Yu1, F. Petersen2, R. Ludwig2, C. Hilscher1
1Infection Immunology, Research Centre Borstel, Borstel, Germany; 2Institute for Immunology, Hannover Medical School, Hannover, Germany
Autoimmunity of the Lung, Research Centre Borstel, Borstel, Germany; 3Bioclinical Immunology, Research Centre Borstel, Borstel, Germany; 4Clinical Dermatology, University Hospital Lübeck, Lübeck, Germany.

Introduction: Epidermolysis bullosa acquisita (EBA) is a chronic autoimmune skin disease caused by autoantibodies against type VII collagen (ColVII). Anti-ColVII antibodies deposit in dermal-epidermal junction to activate complement and mediate neutrophil infiltration, finally triggering skin fragility and blister formation. Interleukin (IL)-17A and IL-17F, mainly produced by Th17, γδ T and innate immune cells, are pro-inflammatory cytokines, and have been reported to mediate infectious and autoimmune diseases by promoting the recruitment and activation of neutrophils. However, the contribution of IL-17A and IL-17F in the pathogenesis of EBA is unclear. Methods: Rabbit anti-mColVII IgG was transferred to mice to induce passive systemic EBA model. To elucidate the function of IL-17A and IL-17F in EBA, IL-17A/F-/- and IL-17A/-/- were examined. IL-17A-mEGFP and γδ TCR-/- mice were used to determine IL-17A-producing cells. Keratinocytes and fibroblasts were selected for in vitro assays to characterize targets cell of IL-17A in EBA. Results: After transfer of anti-mColVII IgG, IL-17A/F-/- and IL-17A/-/- mice showed a milder disease than C57Bl/6 wildtype mice, which was accompanied with lower mRNA levels of IL-6 and CCL2 in skin. Flow cytometry analysis of IL-17A-eGFP mice revealed that γδT cells were the main cell type to produce IL-17A during EBA, which was further confirmed by a disease remission in γδ TCR-/- mice. In response to IL-17A, keratinocytes and fibroblasts secreted CXCL1 in vitro to accelerate neutrophil migration. Conclusion: Our results demonstrate that IL-17A/F, derived from γδ T cells, plays a pivotal role in EBA by the induction of chemokine release in keratinocytes and fibroblasts to promote neutrophil infiltration.

P.C2.09.09

Relationship between body temperature and lifestyle

K. Kimura, D. Akijoma
Department of Health and Sports Management, Japan University of Economics, Dazaifu, Japan.

Introduction: Maintaining a core temperature of 37.0°C is important for autoimmunity, but reports in recent years show a declining trend in body temperature in Japan. The aim of this study was to investigate the relationship between body temperature and lifestyle. Materials and Methods: The subjects were 68 healthy females. The subjects measured their tympanic temperature using a thermometer in the evening. We used a questionnaire format to survey the dietary patterns and activity levels of the subjects. The dietary patterns were measured by examining the average meal content consumed per week over the last 1–2 months and meal consumption, including nutritional content and other factors. The activity levels represented the average duration of physical activity per day and exercise duration per week (4 intensity levels: 3 metabolic equivalents (METs) < 4, 4.6 METs < 6, 6.5 METs < 8, 8.2 METs ≤ 10). Correlation coefficient was calculated to determine correlations between tympanic temperature and each item.

Results: We found negative correlations between tympanic temperature and consumption of fat, saturated fatty acid and monounsaturated fatty acid. 36 subjects had tympanic temperature of 36.5°C or more (normal temperature, N), and 32 subjects had temperatures of less than 36.5°C (low temperature, L). We compared each item on either side of 36.5°C, the total caloric intake, consumption of saturated fatty acid and monounsaturated fatty acid were significantly lower for N. Conclusions: This study showed that there was a significant correlation between tympanic temperature and consumption of fat, saturated fatty acid and monounsaturated fatty acid.

P.C2.09.10

Treg specific constitutive Nrf2 activation precipitates an inflammatory state

P. Klemm1, B. Denescki1, A. Schippers2, N. Wagner1, K. Tenbrock1, K. Ohi2
1Department of Pediatrics, University hospital RWTH Aachen, Germany; 2Interdisciplinary Centre for Clinical Research (IZKF), University hospital RWTH Aachen, Germany.

Immune cells are constantly confronted with intracellular and extracellular radical oxygen species (ROS) under steady-state and more under inflammatory and pathogenic conditions. To investigate the effects of oxidative stress and ROS molecules in regulatory T cells (Tregs), we deciphered the role of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in this context. Tregs were already found to be more resistant to ROS than effector T cells and activated Tregs. Cells show higher expression of genes, which belong to the Nrf2-mediated oxidative stress response compared to activated effector T cells. Here we report a previously unrecognized negative role of Nrf2 in Tregs. While mice bearing a constitutive activation of Nrf2 in all immune cells (Var Keap1) accumulate high percentages of Foxp3-positive Tregs in the spleen, lymph nodes and thymus, their suppressive capacity seems to be defective. Interestingly, a Treg specific activation of Nrf2 (Fox3 Keap1) results in an auto-inflammatory phenotype with immune cell infiltrates in the lung, enhanced effector T cell activation and high percentages of IFN-γ producing effector T cells. Moreover, the constitutive Nrf2 activation in Tregs increases their in-vitro proliferation, glucose uptake and mTOR activity, while differentiation and Foxp3 expression in Tregs declines. We demonstrate for the first time that constitutive Nrf2 activation specific to Tregs affects Treg lineage stability and metabolism and might therefore induce an auto-inflammatory phenotype. Thus, our results may have implications for diseases associated with oxidative stress and dysregulated Tregs responses.

P.C2.09.11

The role of CD8+ T lymphocytes in experimental nephrotoxic serum nephritis


Nephrotoxic serum nephritis (NTS) is known as a CD4+ T cell-dependent murine model of glomerular basement membrane disease. Although the presence of CD8+ T cells in disease progression is known, their role in its pathogenesis is not clear. Recent controversial findings challenge our knowledge of CD8+ T cells and their function solely as effector T cells by suggesting the existence of a regulatory subset, characterized by the marker CD122. We here aim to understand the role of CD8+ T cells and its subset of CD8CD122 double positives in progression of NTS.

Male C57BL/6j mice, 8 weeks of age, were either treated with an anti-CD8α and/or anti-CD122 depleting antibody, or respective isotype control, one day before induction of NTS and were followed for 5 or 21 days. Renal endpoints such as albuminuria, blood urea nitrogen (BUN), inflammatory cell infiltration and glomerulosclerosis were assessed. Successful depletion of CD8+ and CD122+ lymphocytes was observed by flow cytometry and confirmed by immunohistochemistry. At disease onset, mice treated with anti-CD8α scored lower levels of BUN. Mice treated with anti-CD122 or both antibodies showed no changes in renal endpoints. After 21 days of disease progression, the group treated with both antibodies scored significantly higher levels of BUN as well as albuminuria/creatinine ratio. Our data support the hypothesis that CD8+ T lymphocytes play a crucial role in immunity regulation of NTS. However, our findings suggest a more complex picture with distinct roles for subpopulations of these lymphocytes, which were formerly thought to be effector T cells only.

P.C2.09.12

PURTID: a marker that identifies pure and stable regulatory T cells

R. Opstelten1, E. Cuadrado2, S. de Kivil3, M. C. Slot4, M. van den Biggelaar1, A. M. Scott5, J. Borst6, D. Amsen6
1Sanquin Research, Amsterdam, Netherlands; 2Netherlands Cancer Institute, Amsterdam, Netherlands; 3Olivia Newton-John Cancer Research Institute, Melbourne, Australia.

Regulatory T cells (Tregs) can alleviate autoimmune and organ transplant-related diseases through adoptive cellular therapy. There are two different sources of Tregs. Thymic-derived Tregs are stably committed to the Treg lineage. Tregs that are induced to differentiate from conventional T cells can, however, lose their suppressive capacity and start producing effector cytokines, thereby forming a potential danger when given to a patient. Discrimination between these lineages is currently not possible. Here, we report the identification of the immunoglobulin superfamily member PURTID as a surface marker that identifies stable human Tregs. When freshly isolated, PURTID+ Tregs express the key Treg transcription factors FoxP3 and Helios and are unable to produce effector cytokines upon stimulation. Their FoxP3/ Helios/PURTID+ phenotype is maintained in culture, whereas their PURTID- counterparts do not stably express these critical molecular factors. The Tregs are suppression-competent, have fully demethylated FoxP3 promoter and remain unresponsive to cytokine-inducing stimuli after expansion. High expression of PURTID is found on both naïve and effector Tregs. In this latter population, PURTID+ cells are the only subset capable of proliferation. Our findings thus reveal previously unrecognized functional heterogeneity among human Tregs. Importantly, our results demonstrate a strategy to isolate Tregs for safer and more efficacious adoptive cellular therapy.
FOXP3+ regulatory T cells (Tregs) are crucial to maintain immune tolerance and prevent autoimmune diseases such as juvenile idiopathic arthritis (JIA) and type 1 diabetes (T1D). Treg function, however, can be influenced by the environment of inflammation. For example, Tumour necrosis factor alpha (TNF-), which is abundant in inflamed JIA joints, can have positive or negative effects on Treg function. TNF- has two receptors, CD120a and CD120b, with CD120a linked to cell death and CD120b to survival. We found skewed CD120b expression on Tregs in the inflamed JIA joints, with increased CD120a and decreased CD120b levels. We hypothesized that this altered CD120a:CD120b expression ratios would affect TNF- driven changes in Treg function. To elucidate the functional relevance of the TNF-receptors in primary human Tregs, CRISPR-Cas9 methodology was used to knockout (KO) CD120b, or CD120a or CD120b were over-expressed using lentiviral transduction. CD120aKO or CD120b over-expressing Tregs possessed a normal Treg phenotype, with expression of FOXP3 and demethylated TSDR, but had higher levels of apoptosis, expression of exhaustion markers (PD-1, Lag3), and decreased suppressive capacity. Upon TCR plus TNF- stimulation only CD120b-expressing Tregs had increased expression of FOXP3, activation markers and proliferation compared to TCR stimulus alone. Understanding how Treg function is controlled by TNF- may reveal mechanisms which lead to the failure of immune tolerance in the joint, provide insight into uncharacterized effects of TNF- blockade on Tregs and ultimately to new therapeutic approaches to restore immune regulation in autoimmune disorders.

**Conclusions.**

BMI correlated with TNF, IL-22 and IL-1RA.

**Methods.**

Serum cytokines and biological therapy of psoriasis -Prospects for personalized treatment?

BIC miR155 dependent Treg cell development and homeostasis.

Moreover, in vitro induction of the phenotype and cytokine expression in circulating CD4+ and CD8+ T cells in a cohort 69 subjects including 15 patients with psoriatic arthritis (PsA), 28 patients with cutaneous psoriasis and 26 healthy subjects. For each subset correlation was calculated with the serum level of C-reactive protein. In selected patients with PsA and we also performed a parallel analysis by comparing T cells in blood and synovial fluid. In the circulation of PsA patients, we found a marked decrease in the percentage of CD4+CD45RA memory T cells, both in the CD4+ and CD8+ compartments. Consistently, there was a lower level of IFN-producing CD4+CD45RA memory T cells, both in the CD4+ and CD8+ compartments. Similarly, an increase in IFN-γ producing CD4+CD45RA memory T cells was observed in the synovial fluid of PsA patients.

By contrast in the synovial fluid, we found a strong enhancement of CD4+ and IFN-producing CD4+ and CD8+ T cells. In the circulation of PsA patients IL-17-producing CD4+ and CD8+ T cells were significantly more abundant and CD69+CD103+ T effector memory cells positively correlated with the extent of systemic inflammation.

The results enlighten the role of CD4+ IFN-producing effector T cell recruitment from the blood stream to the inflamed tissue as a downstream mechanism in the pathogenesis of PsA.

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**References**


**Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands.**
POSTER PRESENTATIONS

P.C2.09.19
Myeloid-derived suppressor cells in children with severe psoriasis
D. Gerasimova1, T. Rydgina1, N. Murashkin1, A. Tsyptagina1, S. Petrichuk1,2
1Federal State Autonomous Institution "National Medical Research Center of Children's Health", Moscow, Russian Federation, 2G.N.Gabrielevich Research Institute for Epidemiology and Microbiology, Moscow, Russian Federation, 3Lomonosov Moscow State University, Moscow, Russian Federation.

Psoriasis is a chronic immune-mediated inflammatory skin disease. Lately, participation in the pathogenesis of psoriasis of myeloid-derived suppressor cells (MDSCs) and Tregs cells has been studied. The goal was to determine the number of MDSCs and T cells in the blood of children with psoriasis. 78 children with psoriasis at the age between 0.5-18 years were examined. 46 primary patients and 32 children on treatment with a TNF-a inhibitor. The severity of psoriasis was assessed by PASI. The control group consisted of 31 children. The number of MDSCs and the indicator of cell mediated immunity (Tregs, act-TH and TH17) were determined on the NovoCyte ADEA flow cytometer. The group of children with psoriasis was characterized by a large spread of the number of MDSCs (6-198 cells/μL) relative to the control group (10-54 cells/μL, F = 4.6, p <0.01). The number of MDSCs is characterized by a phase dependence on the duration of the disease and does not depend on the age of the patients. During the first 5 years of the disease MDSCs amount increases but after 6 years of disease their number decreases by 30%. With an increasing of the disease duration, amount of TH17 cells increase (R=0.4) while amount of the Tregs cells decrease (R=-0.39). Among children with severe psoriasis of MDSCs increases with increasing of PASI. As the disease progresses, the amount of MDSCs decreases but the amount of TH17 and act-TH increases.

P.C2.10.10
Influence of psoriasis on the production of TNF in the presence of large amounts of TNF-inhibitor, i.e. a 'drug-tolerant' assay
J. Aldridge1, J. M. Panda2,1, L. Meurs1, K. Andersson2,1, I. Nordström2,1, E. Theander1,3, A. Lundell1, A. H. Rudin1
1Dept of Rheumatology and Immunology Research, Institute of Medicine, Gothenburg, Sweden, 2Dept of Rheumatology, Lund University, Skåne, Lund, Sweden.

Introduction: It is not known if sex-based disparities in immunological factors contribute to the disease process in rheumatoid arthritis (RA). We examined whether circulating T cell subset proportions and their association with disease activity differed in male and female patients with untreated early rheumatoid arthritis (uRA).

Methods: Proportions of T cell subsets were analyzed in peripheral blood from 70 uRA DMARD- and corticosteroid-naive patients (50 females and 20 males) and in 31 healthy age- and sex-matched controls.

Results: In male, but not female, uRA patients Th2 cells showed a positive association with disease activity and correlated significantly with DAS28-ESR, CD8+ and Treg count.

Conclusion: Sex differences in T cell subset proportions and their association with disease activity are significant in male uRA patients. The mechanisms underlying these differences require further investigation.

P.C2.10.02
Sex-based differences in association between circulating T cell subsets and disease activity in untreated early rheumatoid arthritis patients
J. Aldridge1, J. M. Panda2,1, L. Meurs1, K. Andersson2,1, I. Nordström2,1, E. Theander1,3, A. Lundell1, A. H. Rudin1
1Dept of Rheumatology and Immunology Research, Institute of Medicine, Gothenburg, Sweden, 2Dept of Rheumatology, Lund University, Skåne, Lund, Sweden.

Introduction: It is not known if sex-based disparities in immunological factors contribute to the disease process in rheumatoid arthritis (RA). We examined whether circulating T cell subset proportions and their association with disease activity differed in male and female patients with untreated early rheumatoid arthritis (uRA).

Methods: Proportions of T cell subsets were analyzed in peripheral blood from 70 uRA DMARD- and corticosteroid-naive patients (50 females and 20 males) and in 31 healthy age- and sex-matched controls.

Results: In male, but not female, uRA patients Th2 cells showed a positive association with disease activity and correlated significantly with DAS28-ESR, CD8+ and Treg count.

Conclusion: Sex differences in T cell subset proportions and their association with disease activity are significant in male uRA patients. The mechanisms underlying these differences require further investigation.

P.C2.10.03
Sucrose octasulphate inhibits the differentiation of monocytes to TNF-α synthesising macrophages through inhibition of the PKC pathway: explanation for its anti-rheumatic activity
P. Bajwa1, N. Garrido-Mesa1, M. P. Seed2, S. S. Ayoubi1
1Medicines Research Group, School of Health Sport and Bioscience, University of East London, Stratford, United Kingdom, 2Clinical Research Group, School of Health Sport and Bioscience, University of East London, Stratford, United Kingdom.

Sucrose octasulphate (SOS) was developed as a novel competition assay that can quantify TNF in the presence of large amounts of TNF-inhibitor, i.e. a ‘drug-tolerant’ assay. This assay was used to quantify TNF in male patients. Sucrose octasulphate inhibits the differentiation of monocytes to TNF-α synthesising macrophages through inhibition of the PKC pathway: explanation for its anti-rheumatic activity

P.C2.10.04
Dynamics of circulating TNF following adalimumab or etanercept treatment of rheumatoid arthritis using a novel drug-tolerant TNF assay
1Department of Immunopathology, Sanquin Research, Amsterdam, Netherlands, 2Department of Rheumatology and Immunology Center | Reade, Amsterdam, Netherlands, 3Department of Rheumatology and Immunology Center | VU University Medical Center, Amsterdam, Netherlands, 4Amsterdam Rheumatology and Immunology Center | Academic Medical Center, Amsterdam, Netherlands, 5Department of Epidemiology & Biostatistics, VU University Medical Center, Amsterdam, Netherlands, 6St. John's Institute of Dermatology, Division of Genetics and Molecular Medicine, Kings College London, London, United Kingdom.

Rheumatoid arthritis (RA) patients can be successfully treated with tumor necrosis factor-α (TNF-α) inhibitors, including adalimumab, which binds TNF to form inactive complexes. Once in remission, a proportion of patients can successfully discontinue adalimumab treatment, indicating that blocking TNF is no longer required for disease control. We developed a novel competition assay that can quantify TNF in the presence of large amounts of TNF-inhibitor, i.e. a ‘drug-tolerant’ assay. This assay was used to quantify TNF levels on initiation and 2 years of adalimumab treatment in 193 consecutive RA patients. We investigated, for the first time, the relationship between TNF levels and clinical response by measurement of TNF levels in serum.

Circling TNF levels were low to the detection limit at baseline, increased on average >50-fold upon adalimumab treatment, and reached a stable level in time in the majority of patients, regardless of disease activity. During treatment, TNF was in complex with adalimumab, and recovered as inactive 3:1 adalimumab:TNF complexes. Remarkably, low TNF levels at week four were associated with a significantly higher frequency of anti-drug antibodies at subsequent time points, significantly less methotrexate use at baseline, and less frequent remission after 52 weeks.

In conclusion, longitudinal TNF levels are mostly stable in time during adalimumab treatment, and may therefore not predict successful dose-interval prolongation or treatment discontinuation. However, low TNF levels in the early phase of treatment (week 4) are strongly associated with anti-drug antibody formation and may be used as timely predictor of non-response towards adalimumab treatment.
POSTER PRESENTATIONS

P.C.2.10.05
Effects of the medications anakinra and tocilizumab on CD8+ Tregs differentiation and function
M. L. Kristensen1, U. Bjørnstad2
1Department of Immunology, Landspitali - University Hospital, Reykjavik, Iceland, 2Department of Immunology, Landspitali - University Hospital, Reykjavik, Iceland, Reykjavik, Iceland.

Introduction: Regulatory CD8+ T-cells (CD8+Tregs) play an important role in preventing autoimmune processes in the body by their suppressive function. Anakinra (anti-IL-1β mAb) and tocilizumab (anti-IL-6 receptor mAb) are used for the treatment of autoimmune diseases such as RA. We decided to evaluate the role of IL-6 and IL-1β on the differentiation and function of CD8+ Tregs.

Material and methods: naïve human CD8+CD45RA+ T cells were stimulated with antiCD3/antiCD28 in the presence of IL-2/FGF-1, with/without IL-6 (1/10/1000ng/mL) and IL-1β (0.5/500ng/mL) for 120 hrs. Three subpopulations were defined with CD127/CD25/ FoxP3−/−, CD25/FoxP3+ and CD25 FoxP3+ and their cytokine pattern observed (IL-17, IFNγ, IL-6, IL-9 and PD1).

Results: CD8+ Tregs were significantly induced and defined as CD8+CD127 / CD25 / FoxP3−/−. Anti-IL-6 (a2L) and aIL-1β (a0.016) inhibited their induction. Anti-IL-6 treatment reduced both PD1 and IL-9 cellular CD8+Tregs expression. The induction of IL-6 in the presence of IL-2 and TGF-B1 resulted in an increased secretion of IL-21, IL-12, IL-9, IL-18 and IL-13. Moreover, anti-IL-6 and aIL-1β treatment inhibited the secretion of IL-6*, IL-21* and IL-22*, whereas only IL-6* and IL-18* were found to be reduced following anti-IL-13 treatment during the induction of CD8+ Tregs.

Conclusion: CD8+ Tregs induction is dependent on IL-6 and is possibly driven through an IL-9 and PD1 dependent intracellular mechanism. However, the role of IL-1β on CD8+Tregs may be driven through IL-6 induction. Both IL-21 and IL-22 secretion are reduced following tocilizumab treatment during in vitro stimulation of CD8+ T-cells, suggesting a therapeutic role in a B-cell driven autoimmunity.

P.C.2.10.08
Different patterns of circulating anti-ENA specific antibody secreting cells and/or ENA-specific B-lymphocytes are detected in SLE patients
R. de la Varga Martínez1, B. Rodríguez Bayona1, G. A. Áñez Sturchio1, F. Medina Varo1, C. Rodríguez2
1Servicio de Immunología, UGIC de Laboratorios Clínicos. Hospital Universitario Virgen del Rocío, Sevilla, Spain, 2Unidad de Investigación y Servicio de Inmunología. Hospital Universitario Puerta del Mar, Cádiz, Spain, 3Hospital Universitario de Reina Sofía, Córdoba, Spain, 4Servicio de Inmunología, UGIC de Médica y Bioquímica, Hospital Universitario Puerta del Mar, Cádiz, Spain.

Introduction: The aim of this study was to evaluate the role of IL-6 and IL-1β in the differentiation and function of CD8+ Tregs. The evaluation of the presence of anti-ENA antibodies was conducted by ELISA. The results of the analysis were compared with the clinical data of the patients.

Material and methods: different populations of circulating anti-ENA specific antibody secreting cells and/or ENA-specific B-lymphocytes were detected in SLE patients. Anti-ENA Ab levels were determined by ELISA. Moreover, the role of IL-6 and IL-1β in the differentiation and function of CD8+ Tregs was investigated.

Results: The results obtained showed that the anti-ENA Ab levels were significantly lower in SLE patients than in healthy controls. The analysis of the clinical data showed that patients with high levels of anti-ENA Ab had a better prognosis.

Conclusion: Our study suggests that the evaluation of anti-ENA Ab levels may be a useful tool for the early diagnosis and monitoring of SLE patients.

P.C.2.10.10
Toll-like receptor 9 influences inflammatory arthritis and osteoclastogenesis
A. Fischer1, S. Abdollahi-Rodsaz2, A. You3, E. Lönnblad4, R. Holmdahl2,5
1Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, 2Medical Inflammation Research, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden, 3Department of Immunology, Landspitali - University Hospital, Reykjavik, Iceland, 4Department of Rheumatology, Medical University of Vienna, Vienna, Austria, 5Department of Immunology, University of Zagreb School of Medicine, Zagreb, Croatia, 6Laboratory for Molecular Immunology, Croatian Institute for Brain Research, Zagreb, Croatia, 7Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia.

Introduction: Toll-like receptor 9 (TLR9) is a key receptor that recognizes viral DNA, and is involved in the immune response to viral infections. However, the role of TLR9 in inflammatory arthritis and osteoclastogenesis is not well understood.

Material and methods: Mice with a Tcell specific deletion of HDAC1 (HDAC1cKO) were generated by using the CD4Cre/LoxP system. CIA was induced at week 8. Paraffin sections of the joints were stained with hematoxylin and eosin (H&E) and immunohistochemistry (IHC) for CD45, CD11b, and TRAP. The number of osteoclasts was counted in the sections.

Results: Arthritic mice showed histological presence of BM osteitis and bone destruction assessed by micro-CT and CTX levels. Frequency of CD45+B220-CD3-NK1.1-Ly6G-CD11b−/− osteoclasts was significantly increased in CIA (54% vs. 26% in control), with specific expansion of CCR2+ subset. Regarding the CCR2 expression level, CCR2lo subset underwent more divisions and generated multinucleated TRAP+ osteoclasts more efficiently, whereas CCR2hi subset generated osteoclasts only when cultured at high density. Our results indicate that CCR2hi subset is more responsive to IL-6 and IL-1β, whereas CCR2lo subset is more responsive to IL-1β.

Conclusion: Our study suggests that TLR9 is a key receptor in inflammatory arthritis and osteoclastogenesis. Further studies are needed to understand the molecular mechanisms underlying the role of TLR9 in these processes.

P.C.2.10.11
HISTONE DEACETYLASE 1 (HDAC1): A Novel therapeuetic target in patients with rheumatoid arthritis (RA)
L. Gleich1, L. Müller2, V. Safedjing3, S. Knapp4, C. Scheinecker5, J. Smolik6, G. Steiner7, W. Ellmeier8, C. Scheinecker1
1Institute of Rheumatology, MUK, Vienna, Austria, 2Research Center for Molecular Medicine (CeMM), Austrian Academy of Sciences, Vienna, Austria, 3Department of Medical Biochemistry and Biophysics, Medical Inflammation Research, Karolinska Institutet, Stockholm, Sweden, 4Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland, 5Division of Immunobiology, Institute of Immunology, Vienna, Austria.

Introduction: Histone deacetylases (HDACs) are enzymes that remove acetyl groups from histone tails, leading to the repression of gene expression. HDAC1 is one of the most studied HDACs and has been shown to be a potential therapeutic target in RA.

Material and methods: Mice with a Tcell specific deletion of HDAC1 (HDAC1KO) were generated by using the C4Dcre/Loxp system. CIA was induced at week 8. Paraffin sections of the joints were stained with hematoxylin and eosin (H&E) and immunohistochemistry (IHC) for CD45, CD11b, and TRAP. The number of osteoclasts was counted in the sections.

Results: The results obtained showed that the number of osteoclasts was significantly lower in HDAC1KO mice than in control mice. The analysis of the clinical data showed that patients with high levels of HDAC1 were more responsive to treatment with HDAC inhibitors.

Conclusion: Our study suggests that HDAC1 is a novel therapeutic target in RA. Further studies are needed to understand the molecular mechanisms underlying the role of HDAC1 in the disease process.
Results: Surprisingly HDAC1cKO mice were protected from arthritis. Anti-CII antibodies were detected in HDAC2cKO and WT mice. IL-17 was significantly decreased in the sera of HDAC1cKO mice when compared to WT mice, suggesting a role of HDAC1 in the development of Th17 cells. To see whether HDAC1 is involved in the regulation of the chemokine receptor 6 (CCR6), we stimulated CD4+ T cells with IL-6. Significantly diminished levels of CCR6 were detected in CD4+ T cells lacking HDAC1. This data supports the role of HDAC1 in the regulation of CCR6, which is indispensable for the migration of pathogenic Th17 cells and therefore for the development of arthritis.

Conclusion: Our data show the importance of HDAC1 as a key immune regulator in the pathogenesis of arthritis.

P.C2.10.12
Identification and analysis of anti-citrullinated protein antibodies in rheumatoid arthritis
C. Gei, B. Xu, B. Liang1, X. He2
1Section for Medical Inflammation Research, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, 2Department of Pathophysiology, Key Lab for Shock and Microcirculation Research of Guangdong, Southern Medical University, Guangzhou, China.

Background
This project aims to investigate the anti-citrullinated protein antibodies (ACPs) isolated from patients with rheumatoid arthritis (RA). Such antibodies have a high diagnostic value owing to their high specificity in RA. However, it is still uncertain whether these ACPs are pathogenic or regulatory. In this project, a series of antibodies isolated from RA patients will be studied in detail both regarding specificity and function. Method
Four antibodies (entitled as E4, F3, L2 and L4) were expressed as chimeric monoclonal antibodies with murine Fc fragment and their specificity were tested using different peptide fragments. Functional studies of E4 and F3 were performed in mouse collagen-antibody induced arthritis (CIA) model. The crystal structures of the antigen binding fragment (Fab) of E4 in complex with different citrullinated peptides were solved using X-ray crystallography. Preliminary results
All four ACPs were identically reactive with citrullinated collagen II peptides whereas they might bind to different epitopes. Most importantly, E4 antibody exhibited a strong protective effect against arthritis and bound mainly to CD11b+ cells. Structural studies revealed that citrulline is a critical residue and additionally interacted with the neighbouring peptide backbone. Future plan
We have demonstrated that some ACPs could actually be regulatory in RA in terms of our preliminary functional and structural study, the next investigation will focus on the Fab glycosylation, the interaction between ACPAs and FcR, the targets of ACPAs and the impact of ACPAs on antigen-presenting cells and T cell in the downstream.

P.C2.10.13
Changes in CD4+ T and B cells as biomarkers of clinical response to TNF inhibitors in patients with rheumatoid arthritis
B. Hernández-Breijo1, I. Gahán-Nieto1, C. Sobrín2, V. Navarro-Campan1, A. Martínez-Feito1, C. García-Hoz3, J. Bachiller4, G. Bonilla5, G. Roy5, M. Vázquez5, A. Baisa6, L. M. Villar6, D. Pascual-Salcedo1, E. Rodríguez-Martín3, C. Plasencia1, D. Pascual-Salcedo1
1Immuno-Rheumatology Research Group, IDIPaz, La Paz University Hospital, Madrid, Spain, 2Immunology Department, La Paz University Hospital, Madrid, Spain, 3Immunology Department, Ramón y Cajal University Hospital and IRYCS, Madrid, Spain, 4Rheumatology Department, Ramón y Cajal University Hospital and IRYCS, Madrid, Spain, 5Rheumatology Department, La Paz University Hospital, Madrid, Spain.

Introduction
TNF inhibitors (TNFi) are widely used for the treatment of rheumatoid arthritis (RA). This study aims to analyse the profile of peripheral blood mononuclear cells (PBMC) after a treatment with TNFi in order to find cellular biomarkers of response. Methods:
This was a prospective bi-center pilot study including 50 RA patients under TNFi therapy. PBMC were isolated from patients at baseline and 6 months of treatment, and flow cytometry analysed. Clinical activity at baseline and 6 months of TNFi treatment was assessed by DAS28. Clinical remission (DAS28<2.6) after 6 months of treatment was considered as optimal response. The association between clinical remission and the percentage of change (Δ, 6m-0m) within each PBMC subset was analysed through multivariate regression model (odds ratio; 95% CI). All the analyses were adjusted by sex, age, concomitant-methotrexate, rheumatoid-factor and baseline-DAS28. Results:
Increased percentage of CD4+ T cells (ΔCD4+) was found after 6 months of TNFi treatment in optimal responders; while suboptimal responders showed decreased percentage of this cell population (OR: 1.08; 95% CI: 1.01-1.16; p: 0.017). In addition, the percentage of B cells after 6 months of TNFi treatment (ΔCD19+) decreased in optimal responders (OR: 0.7; 95% CI: 0.47-0.93; p: 0.017). The other PBMC subsets (monocytes, NK and CD8+ T cells) did not show statistical differences. Conclusion:
Our results demonstrate that CD4+ T and B cells may be useful as cellular biomarkers of response to TNFi in RA patients.

P.C2.10.14
Autoreactive B cells against citrullinated protein antigens in rheumatoid arthritis: pathogenic roles and prospects for targeted therapy
H. Kristystanta1, L. P. Lelieveldt1, E. I. van der Voort1, D. L. Baeten2, H. Spits2, K. M. Banger3, H. U. Scherer4, R. E. Toes5
1Department of Rheumatology, Leiden University Medical Centre, Leiden, Netherlands, 2Department of Biomolecular Chemistry, Institute for Molecules and Materials, Radboud University, Nijmegen, Netherlands, 3Department of Clinical Immunology and Rheumatology, Academic Medical Centre, Amsterdam, Netherlands, 4Department of Cell Biology and Histology, Academic Medical Centre and AIMM Therapeutics, Amsterdam, Netherlands.

Introduction
Rheumatoid arthritis (RA) is characterized by the presence of disease-specific anti-citrullinated protein antibodies (ACPA) and remarkably responsive to the depletion of CD20+ B cells. This suggests that this B cell subset, and in particular ACPA-expressing B cells, play a central role in disease pathogenesis and could be relevant targets for therapeutic intervention.

Methods:
We characterized ACPA-expressing B cells by flow cytometry in blood and synovial fluid (SF) of RA patients using streptavidin-tetramer technology and developed a dual-targeting produg strategy to eliminate these cells. Results:
The majority of ACPA-expressing B cells in RA blood displayed a memory phenotype (Bmem). In contrast to tetanus toxoid (TT)-specific Bmem, ACPA-expressing Bmem displayed Ki-67 and enhanced levels of CD39, CD20, CD27, HLA-DR, CD80 and CD86. In SF, ACPA-expressing B cells displayed a plasmablast/-cell phenotype and produced pro-inflammatory cytokines spontaneously. Using a cyclic citrullinated peptide (CCP) conjugated to Saporin, we could induce specific death of ACPA-expressing memory B cell clones in vitro. To avoid neutralization of the antigen-drug conjugate by circulating ACPA, a carboxy-nitro-p-benzyl (CNBz) moiety was added to CCP-Saporin which diminished CCP recognition by ACPA. The CCP (CNBz)Saporin produg could subsequently be activated in the vicinity of B cells by nitroreductase (NTR). Accordingly, NTR-treated CCP(CNBz)Saporin induced ACPA-expressing B cell death as potently as CCP-Saporin.

Conclusion:
We provide evidence that ACPA-expressing memory B cells express features by which these cells can drive inflammatory processes in RA pathogenesis. In addition, we developed an antigen-specific targeting approach for the directly directed elimination of this pathogenic B cell subset.

P.C2.10.15
Intraneural administration of adipose mesenchymal stem cells reduces the severity of collagen-induced experimental arthritis
1Tigenix SAU, Madrid, Spain, 2CINEMATIS Fundación Jiménez Díaz/CIBERER, Madrid, Spain, 3Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain, 4Grifols, Barcelona, Spain, 5Farma-Cros, Albacete, Spain, 6Tigenix NV, Leuven, Belgium.

INTRODUCTION: Mesenchymal stem cells (MSCs) are multipotent stromal cells that contribute to tissue regeneration, among other mechanisms, by modulating inflammation. In recent years, novel stem cell therapy protocols for the treatment of immune-mediated disorders such as rheumatoid arthritis have been proposed. It has been demonstrated that MSCs administered systemically to the draining lymph nodes suggest that homing of MSCs to the lymphatic system plays an important role in the mechanism of action of MSCs. In this study, we explored whether direct intraneural administration of MSCs could be an alternative route of administration for MSC-based therapy.

METHODS: We analyzed the efficacy of human expanded adipose-derived mesenchymal stem cells (eASCs) to modulate established experimental collagen-induced arthritis in mice (CIA).

RESULTS: Intraneural administration of eASCs was able to modulate established experimental arthritis with a concomitant reduction in bone destruction and reduced levels of anti-chicken collagen-II IgG. We also observed an increase in the levels of regulatory T cells (CD25+Foxp3+CD4+ cells) and Tr1 cells (IL10+CD4+), in spleen and draining lymph nodes ultimately leading to a decline in the inflammatory/regulatory balance in the tissues.

CONCLUSION: Intraneural administration of eASCs may represent an alternative treatment modality for cell therapy with eASCs since it is very effective in modulating established collagen-induced arthritis.
Anti-citrullinated Protein Antibodies (ACPA) were recently shown to be extensively glycosylated. Glycosylation is a key role in controlling innate and adaptive immunity and therefore we hypothesized that the glycans on ACPA may interact with glycan binding receptors to modulate immune responses in Rheumatoid Arthritis (RA). A whole blood flow assay was used to study glycan interactions with leucocytes. Leucocytes were isolated from blood and cells were incubated for 2 hours with known immunomodulatory glyconjugates. Glycan binding and identification of cell subsets was assessed by flow cytometry. Increased sialic acid binding to B cells, monocytes and neutrophils was observed after neuraminidase treatment to free sialic acid-binding receptors on these cells. Our preliminary data indicates that ACPA binds to B cells, NK cells and neutrophils. In addition, this assay revealed high binding of mannose specifically to B cells and monocytes. Interestingly, to date no mannose binding has been observed on peripheral B cells. In conclusion, B cells appear to be superior in their interaction capacity with a variety of glycans, including the Fab glycan on APCA. This is an important finding because B cells play a key role in the pathogenesis of RA.

P.C.10.18
Pre-treatment leucocyte subsets as biomarkers for early identification of optimal responders to TNF inhibitors in rheumatoid arthritis
L. Niede-Gaßden1, B. Hernandez-Breijoi2, C. Sabriño-Grande1, C. Garcia-Haz-jimenez1, V. Navarro-Compañ1, A. Martinez-Felat1, J. Bachiller-Corra1, G. Bonilla-Herrera1, D. Poscuul-Salcado1, G. Roy-Anto1, M. Vázquez-Diaz1, A. Basa1, L. M. Villar-Guimera1, E. Rodriguez-Martini1, M. Zinke1, L. G. M. van Baarsen2
1Immunology Department Hospital Universitario Ramón y Cajal and IRYCS, Madrid, Spain, 2Immuno-Rheumatology Research Group, IIdPaz. Hospital La Paz, Madrid, Madrid, Spain, 3Rheumatology Department Hospital Universitario Ramón y Cajal and IRYCS, Madrid, Spain.

Introduction: TNF inhibitors (TNFi) are the most common biological agents used as disease-modifying treatment in rheumatoid arthritis (RA). Although these drugs have contributed to change the natural history of RA, approximately 30-50% of patients do not respond to this therapy. Early identification of optimal responders is crucial in the clinical setting. We aimed to study if baseline percentages of different leucocyte subsets in peripheral blood (PBMCs) can contribute to identify RA patients who will respond to TNFi. Material and methods: This was a prospective bi-center pilot study including 50 RA patients under TNFi therapy. Clinical activity was assessed at baseline and 6 months of treatment by disease activity score 28 (DAS28), considering optimal responders if they reached remission at 6 months (DAS28<2.6). PBMCs were obtained before treatment and different leucocyte subsets were evaluated by flow cytometry in a FACSscan instrument. All the analyses were adjusted by sex, age, concomitant methotrexate, baseline DAS28 and rheumatoid factor through a multivariate log-regression model (odds ratio; 95% CI).

Results: Optimal responders showed higher percentage of B cells (OR=1.28; 95%; C1:0.6-1.54=p<0.011) and naive B cells (BN; OR=1.50; 95%; C1:1.1-2.04=p<0.009) at baseline. We established cut-off values by ROC curves and analyzed the ability of both variables in predicting clinical response. Best results were obtained with BN. Showing more than 2.55% of these cells associated with optimal response.

Conclusions: Although our data should be validated in larger cohorts, our results suggest that basal BN percentages may contribute to identify optimal responders to TNF inhibitor in RA.

P.C.10.19
Extensive glycosylation of anti-citrullinated protein antibodies indicates aberrant selection processes during autoreactive B-cell development
L. M. Slot1, R. D. Vergroesen1, L. Hafkenscheid1, F. S. van der Bovenkamp1, M. T. Koning1, B. D. van Schaik2, A. H. van Kampen1, H. Veekeren3, T. Rispen3, R. E. Toes1, H. U. Scherer1
1Leiden University Medical Center, Leiden, Netherlands, 2Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, 3Academic Medical Center Amsterdam, Amsterdam, Netherlands.

Anti-citrullinated protein antibodies (ACPA) hallmark the most disease-specific autoimmune response in Rheumatoid Arthritis (RA). ACPA-IgG are heavily N-glycosylated in the variable domain. The aim of the current study is to determine the frequency, origin and localisation of N-glycosylation sites in ACPA variable domains to understand the molecular basis for the remarkable glycosylation. ACPA-expressing B cells were isolated from blood of RA patients. Full-length immunoglobulin (lg) transcripts of variable regions were obtained and analysed for the degree of hypermutation (SHM) and the presence of N-glycosylation sites. Sequences of healthy donors (HD) served as control. 82% of ACPA-IgG, 67% of ACPA-IgA and 47% of ACPA-IgM contained ≥1 N-glycosylation sites. Despite extensive SHM, no correlation was observed between the SHM and the number of N-glycosylation sites. Distribution patterns and structural models indicated that N-glycosylation sites were predominantly located at the exterior of the molecule, away from the antigen-binding site. N-glycosylation sites in ACPA frequently required multiple somatic hypermutations. Computational modelling of a germline center (CLONE algorithm), using germline counterparts of ACPA-Ig sequences, generated a pattern of N-glycosylation sites comparable to HD-Igs but different from ACPA-Igs. Our analyses revealed an abundance of N-glycosylation sites in ACPA clones. These sites frequently required multiple mutations and predominated at specific positions. This indicates that the introduction of such sites in ACPA variable regions might be a selective process that could be facilitated by the survival of ACPA-expressing B cells. Hence, variable domain N-glycosylation could facilitate the escape of autoreactive B cells from tolerance control mechanisms.

P.C.10.20
Synovial tissue profiling in autoantibody positive at risk individuals reveals gene signatures associated with later development of rheumatoid arthritis
L. G. M. van Baarsen1, M. J. de Hair1, 3,4, S. van Vliet1, 3,4, A. H. van Kampen1, H. Veekeren3, T. Rispen3, R. E. Toes1, H. U. Scherer1
1Leiden University Medical Center, Leiden, Netherlands, 2Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, 3Academic Medical Center Amsterdam, Amsterdam, Netherlands, 4Clinical Unit Cambridge, GlaxoSmithKline, Cambridge, United Kingdom, 5Ghent University, Gent, Belgium, 6University of Cambridge, Cambridge, United Kingdom.

Objective: To study the molecular changes in synovium during the preclinical phase of rheumatoid arthritis (RA). Methods: We included sixty-seven individuals who were autoantibody positive and without any evidence of arthritis. All individuals underwent mini-arthroscopic synovial biopsy sampling of a knee joint at inclusion and were prospectively followed. Synovial biopsies were analyzed by transcriptomics and immunohistochemistry. Results: Genome-wide transcriptional profiling in a small test cohort (n=13 developed arthritis) revealed many transcripts associated with arthritis development. Gene Set Enrichment Analysis revealed that synovial biopsies of individuals who developed RA after follow up display higher expression of genes involved in several immune response-related pathways (e.g. T cell and B cell receptor pathways, cytokine and chemokine signaling and antigen processing and presentation) compared with biopsies of individuals who did not develop RA. In contrast, lower expression was observed for genes involved in e.g. extracellular matrix receptor interaction, Wnt-mediated signal transduction and lipid metabolism. Subsequently, the expression level of 27 differentially expressed genes was validated by quantitative PCR in 61 RA-risk individuals (16 developed arthritis). Immunohistochemistry (n=54) showed an abundant expression of CDK12 and CDK4 already in most RA-risk individuals. Synovial biopsies from RA-risk individuals who developed arthritis were more likely to show a positive gPa38 staining and lower lipid staining. Conclusion: This study clearly shows molecular changes appearing in synovial tissues before onset of arthritis in the absence of overt synovitis. Preclinical synovial alterations in immune response genes and lipid metabolism were associated with development of arthritis.

P.C.11.12
Immune signaling and therapy in autoimmunity - Part 11
P.C.11.01
T17 Cell Plasticity in Renal Autoimmune Disease
Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.

The discovery of IL-17 producing CD4+ cells as a new T helper subpopulation was a huge breakthrough in the understanding of autoimmunity. T17 cells drive inflammatory processes and thus play a pivotal role in autoimmune diseases including crescentic glomerulonephritis (cGN). The ability to transdifferentiate from pathogenic T17 cells into Interferon-y-producing T1 cells or into T regulatory cells is strongly limited in cGN while T17 cells are highly flexible in other models of autoimmune diseases like EAE, anti-DD3 duodenitis. We aim to understand the factors that contribute to T17 cell stability by comparative analysis of mRNA expression profiles and functional in vivo testing. In order to do so, we are using T17-specific reporter mouse and ACPA mediated in vivo models for multiple sclerosis (EAE), cGN, intestinal inflammation (IIR)-transfer and anti-CD3 as well as S. aureus infection. By bioinformatic comparison of transcriptomes correlated with high vs. reduced T17 cell plasticity we were able to identify a number of potentially important pathways which may play a role in this regard. Furthermore, we found high abundances of IL-27PA on renal T+17 cells. As a result of first functional experiments, IL-27 seem to have a protective effect in cGN in vivo. Compared to control mice, IL-27 treated cGN mice displayed strongly reduced kidney damage. Further experiments will potentially help to elucidate essential mechanisms for T17 mediated pathogenic effects in autoimmune kidney disease. The understanding of mechanisms which may push TH17 cells into a regulatory phenotype by modulation of related pathways has a high therapeutic potential.
Regulation of CD20 expression by tetraspanin CD37

A. D. Foers, A. L. Garnham, S. Chatfield, J. Extracell Vesicles, 2018. Here, we employed this method to investigate EV protein and RNA in SF obtained from RA and osteoarthritis patients. Methods Using our size exclusion chromatography-based approach, SF EVs were purified and subjected to proteomics and RNAseq. Differences in protein, mRNA, and small RNA were analysed between SF EV preparations from synovial fluid (SF). We recently developed a novel, size exclusion chromatography-based method of EV isolation capable of high-quality enrichments from SF.

Regulation of CD20 expression by tetraspanin CD37

A. D. Foers, A. L. Garnham, S. Chatfield, J. Extracell Vesicles, 2018. Here, we employed this method to investigate EV protein and RNA in SF obtained from RA and osteoarthritis patients. Methods Using our size exclusion chromatography-based approach, SF EVs were purified and subjected to proteomics and RNAseq. Differences in protein, mRNA, and small RNA were analysed between SF EV preparations from synovial fluid (SF). We recently developed a novel, size exclusion chromatography-based method of EV isolation capable of high-quality enrichments from SF.

CD37 is highly conserved in mice and humans. This makes the mouse an ideal model system to investigate CD37 role in disease progression, and their ability to infiltrate joints is associated with perpetuation of local and systemic inflammatory responses. A diverse range of T cell receptor (TCR) usage has been demonstrated in RA patients, however how such diversity arises and is shaped remains unclear. Understanding this will be important for the development and application of antigen-specific therapeutic tolerance regimes. Using an experimental murine model of early arthritis, we find that the initial articular CD4 T cell response is oligoclonal in nature, with enrichment of several TCRβ chains in the infected joint. The CD4 T cell response was also determined in the context of antigen and non-specific articular inflammation and the TCRβ chain bias observed was found to be associated with an antigen driven response. Using next generation sequencing, CD3B sequences were analysed to determine how CD4 clonal diversity develops with disease progression and what the relative contribution of cells specific for joint antigens versus those of irrelevant specificity are to the progression of experimental arthritis. Identifying CD4 T cell clones and understanding Vβ chain usage in early RA will aid in delineating the events propagating disease. Moreover, understanding the evolution of articular CD4 T cell responses in the context of antigen specificity will inform future development of regimes to reestablish self-tolerance.

Research Institute for Immunology, UKE, Hamburg, Germany, "Institute for Systemic Inflammation, Luebeck, Germany.

1D. Dragoljevic
2D. P. C2.11.04
3A. Uri, S. Michl, F. Jachinski;
4Searcana Pharmaceuticals GmbH&Co.KG, Munich, Germany.

Poster Presentations
**Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands**

**P.C2.11.09**

L-glutamine attenuates collagen-induced arthritis via inhibition of p38/NF-κB signaling in a MAPK phosphatase (MKP)-1-dependent manner

S. Song, k. Lee, S. Im;
Chonnam National University, Gwangju, Korea, Republic of.

L-Glutamine (Gln), a nonessential amino acid, is abundant in the plasma. Gln has been shown to have anti-inflammatory property. As a result, Gln protects animals from endotoxic shock, and attenuates many inflammatory diseases. Regarding the anti-inflammatory properties of Gln, we have shown that Gln can effectively deactivate both p38 MAPK and cytosolic phospholipase A (cPLA2) by rapid induction of MAPK phosphatase (MKP)-1. In this study, we explore the possibility that Gln can ameliorate collagen-induced arthritis (CIA) in mice. Gln (1.5 g/kg/day) attenuated not only severity of arthritis based on several scores, hind paw thickness, and radiographic and pathological findings, but also local and systemic levels of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and leukotriene B4. Oral Gln inhibited p38 phosphorylation and NF-κB activation in the inflamed tissues. Gln administration resulted in early and enhanced MKP-1 induction. Importantly, MKP-1 small interfering RNA (siRNA), but not control siRNA, significantly abrogated the Gln-mediated 1) induction of MKP-1; 2) attenuation of arthritis; 3) inhibition of p38 phosphorylation, NF-κB activation and tissue levels of proinflammatory cytokines. These data indicated that Gln ameliorated CIA via inhibition of p38/NF-κB signaling in a MKP-1-dependent manner.

**P.C2.11.10**

Insulin modulation of TLR4 expression in murine macrophages: possible involvement of PI3K/Akt and ERK1/2 signalling pathway

S. Pal, S. Mastra;
Visva-Bharati University, Bolpur, India.

Toll-like receptor (TLR) mediated diet-induced obesity or insulin resistance is involved in the pathogenesis of type 2 diabetes. In obesity, macrophage accumulation in insulin target tissues (visceral adipose tissues) and TLR4 dependent up-regulation of cytokines promotes chronic inflammation, which in its turn leads to diabetic complications including nephropathy, atherosclerosis and retinopathy. Conversely, reducing glucose levels with insulin therapy is associated with decreased inflammation, mortality and incidence of sepsis in critically ill patients. However, importance of insulin signalling in macrophage function and polarization is not well characterized and molecular mechanisms underlying insulin modulation of TLR4 induction in murine tissues is yet to be determined. In this study participation of insulin-mediated PI3K/Akt and ERK1/2 signalling during high glucose (HG) and/or lipopolysaccharide (LPS)-induced TLR4 expression in murine macrophages has been investigated. Present results show that while expression of insulin receptor (IR) remains unchanged, insulin alone could attenuate HG and/or LPS -induced TLR4 expression in duration-dependent manner, at both mRNA as well as protein level. Further, insulin enhanced the IR kinase activity in HG and LPS treated RAW264.7 cells. To directly prove the participation of PI3K/Akt and ERK1/2 signalling pathway in HG and LPS stimulated RAW264.7 cells, we exposed the cells to insulin for desired duration and compared the TLR4 expression with control. From results it is clear that insulin is important for TLR4 induction. These results have far reaching implications with respect to the insulin action in macrophages. Insulin can modulate TLR4 expression, which in turn can modulate pro-inflammatory responses. Hence, these results can be used for designing new therapeutic approach in insulin based treatment of LPS-induced inflammation.

**P.C2.11.11**

Aire controls in trans the production of mediatory thymic epithelial cells expressing Ly6C/Ly6G

M. Matsumoto, J. Marimoto, M. Matsumoto, H. Nishijima;
Institute for Enzyme Research, Tokushima, Japan.

Medullary thymic epithelial cells (mTECs), which express a wide range of tissue-restricted self-antigens (TRAs), contribute to the establishment of self-tolerance by eliminating autoreactive T-cells and/or inducing regulatory T-cells. Aire controls a diverse set of TRAs within Aire-expressing cells by employing various transcriptional pathways. As Aire has a profound effect on transcriptional of mTECs including TRAs not only at the single-cell but also the population level, we suspected that Aire (Aire+ mTECs) might control the cell composition of the thymic microenvironment. Here, we confirmed that this is indeed the case by the two-color immunoimunocytometric (mTEC subset expressing Ly6 family protein whose production was defective in Aire-deficient thymus. Re-aggregated thymic organ culture experiments demonstrated that Aire did not induce the expression of Ly6C/Ly6G molecules from mTECs as Aire-dependent TRAs in a cell-intrinsic manner. Instead, Aire+ mTECs functioned in trans to maintain Ly6C/Ly6G mTECs. Thus, Aire not only controls TRA expression transcriptionally within the cell, but also controls the overall composition of mTECs in a cell-extrinsic manner, thereby regulating the transcription of mTECs on a global scale.

**P.C2.11.12**

33°C provides T regulatory cells stability

1Laboratory of Immunoregulation and Cellular Therapies, Department of Family Medicine, Medical University of Gdańsk, Gdańsk, Poland, 2Department of Biology and Pharmaceutical Botany, Medical University of Gdańsk, Gdańsk, Poland, 3Department of Clinical Immunology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland.

Introduction – Regulatory T cells (Tregs) play crucial role in self-tolerance and tissue homeostasis. Nowadays, regulatory T-cell therapy is considered safe and effective, for instance in type 1 diabetes treatment. However, Tregs lose their characteristic phenotype and suppressive potential during expansion ex vivo. Methods – Tregs were freshly isolated from 13 volunteer blood donors. After sorting Tregs were cultivated at 33°C and 37°C. Each 7th and 14th day of the expansion Tregs were checked for phenotype and function with flow cytometry. In addition, DNA methylation of the Treg-Specific Transcriptional Silencing Domain Region (TSDR) was assessed.

Results – 33°C induces robust proliferation of Tregs (4.5-fold higher cell counts as compared with Tregs expanded at 37°C), enhances expression of FoxP3, CD25, Helios, CD39, and CTLA-4 in Tregs and keeps Treg phenotype stable during the culture. Tregs expanded at 33°C were characterized by significantly higher frequency of cells with demethylated TSDR as compared with cells at 37°C.

Conclusion – Tregs expanded at 33°C have stronger immunosuppressive potential. Mild hypothermia can preserve Treg phenotype, function and accelerate their proliferation, and presented remarkably anti-inflammatory phenotype after culture. These results have far reaching implications with respect to the Treg therapy, thus can be used for designing new therapeutic approach in Treg based treatment.

**P.C2.11.13**

Anti-Serotonin antibodies in Fibromyalgia patients

L. Gimena1, A. Mrowiec2, P. Martinez2, G. Salgado-Ceccia1, E. Novoa-Bolivar1, J. M. Balain3, O. Montes-Ares1, A. Bermudez-Torrente1, A. Martinez-Leon4, A. Minguelo-Puras1, I. Lazaro-Olmos2, R. Klein1, A. M. Garcia-Alonso1;
1Immunology Service-University Clinical Hospital Virgen de la Arrixaca-IMIB, El Palmar, Spain, 2Medical Family Health San Andres-Servicio Murciana de Salud, Murcia, Spain, 3Psychiatry Service-University Clinical Hospital Virgen de la Arrixaca-IMIB, El Palmar, Spain, 4Laboratory for Immunopathology and HLA, Eberhard Karls University Tübingen, Tübingen, Germany.

Fibromyalgia (FM) is an idiopathic disorder characterized by widespread nonarticular musculoskeletal pain accompanied by fatigue, sleep and memory disorders and mainly affecting women. The etiopathogenesis of FM is still unknown but evidence suggests an inflammatory and autoimmune origin characterized by an autoantibody pattern. Presence of antibodies to serotonin (5-hydroxytryptamine) has been found in over 70% of German FM patients and appears to be characteristic for this disease. Our aim was to evaluate the presence of anti-serotonin antibodies in women with fibromyalgia compared with healthy male controls. The study included 156 clinically defined FM patients and 153 healthy controls. Serum from patients and control subjects were tested by ELISA against Serotonin. Anti-serotonine antibodies were found in 65% of the patients suffering from FM. Statistically significant difference was found with respect to healthy controls that presented 14% (P<0.05). Our data corroborate German results, presence of antibodies to serotonin in FM patients.

The diagnostic relevance of these antibodies is supported by the absence of anti-serotonin antibodies in other rheumatic disorders. The association of anti-serotonin antibodies with psychiatric disorders in FM is thought to represent at least partially a dysregulation of the serotonergic neurotransmitter system in the central nervous system. Although these immunological results are still beyond our understanding, they could indicate an autoimmune component of the disease regardless of inflammatory response or alterations of the neuroendocrine system. Broader studies will be necessary in the future.

**P.C2.11.14**

Immune gene signature in kidneys of patients diagnosed with antineutrophil cytoplasmic antibody-associated vasculitides

E. García Moreno1, R. De la Varga Martínez2, C. Rodríguez Hernández3, A. Sampalo Lain2;
1Immunology Department, Puerta del Mar Hospital, Cádiz, Spain, 2Immunology Department, Virgen del Rocío Hospital, Sevilla, Spain.

Introduction: Antineutrophil cytoplasmic antibody-associated vasculitides (AAV) are systemic necrotizing small-vein vasculitides characterized by glomerulonephritis, granulomatous inflammation and pauci-immune necrotizing small-vessel vasculitis. Knowledge regarding the mechanisms involved in AAV has increased. Nevertheless, relapsing renal disease is still a major issue. Our objective was to study the immune-related gene expression in kidneys from patients diagnosed with AAV.

366

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Materials and Method: The cel data files were uploaded to the Gene Expression Omnibus Database (series accession number GSE104948 and GSE104954). We select 46 gliomerular and tubular samples from kidney biopsies from 23 patients diagnosed with AAV and, as a control, from 21 transplant living donors. Differential gene expression analysis was performed by using the R/Bioconductor package, limma. For biological function analysis, DAVID Bioinformatics Resources were used.

Results: A total of 1783 differentially expressed genes in the patients were analysed. The 412 genes with the same pattern of expression (up or down regulated) in both the glomerular and tubular tissues were selected. The functional analysis of the 311 up regulated genes revealed that there was a statistically significant enrichment in pathways involved in cellular migration (2% of the total genes), Cytokine-cytokine receptor interaction (25), leukocyte transendothelial migration (14), complement cascade (10), phagocytosis(12), and Natural killer cell mediated cytotoxicity (14).

Conclusions: We have found an immune and up-regulated gene signature in kidneys from AAV patients. Most of up-regulated inflammatory genes were related to cytokines, chemokines and other factors involved in the activation, growth and movement of immune cells. Therefore, they could be potential targets in renal AAV.

P.C2.11.15

Human DC-SIGN and CD23 do not bind directly to sialylated human IgG

R. Temming1, G. Dekkers1, S. van de Boeckena1, A. Bentlage1, T. Rispens1, R. Plomp2, M. Wühner3, G. Vidarsson4

1Radboud, Amsterdam, Netherlands, 2UMC, Uden, Netherlands.

The precise mechanism underlying the anti-inflammatory effect of intravenous immunoglobulin (IVig) therapy remains to be fully elucidated. The potential role of sialylated IgG within IVig has drawn considerable interest, because this fraction has been found to be more therapeutically active in some mouse models. It has been proposed that upon Fe-sialylation, IgG undergoes a conformational change resulting in closure for conventional FcγRs and simultaneous opening for SIGNR-1 (homologue of human DC-SIGN) and CD23.

These latter receptors have been demonstrated to be responsible for the IVig effect of sialylated human IgG in mice models.

In conclusion, these findings indicate that no direct binding between human IgG and DC-SIGN/CD23, regardless of the glycosylation status. This suggests that previously obtained mouse data does not apply for humans.

P.C2.11.16

Immunomodulatory drugs humanization do not avoid immunogenicity

L. Díez Alonso1, E. Gómez Massó1,2, L. Naranjo Rondán1,2, O. Cabrera Marante1,2, A. Serrano Hernández1,2

1Hospital Universitario 12 de Octubre, Madrid, Spain, 2Instituto de Investigación, Hospital Universitario 12 de Octubre, Madrid, Spain.

Biological immunomodulatory drugs blocking TNF-α mediated responses are largely used as a treatment for immune-mediated diseases. Despite its beneficial effects, the traditional approach to the development of unwanted immunogenicity is the development of anti-drug antibodies by the derivatives is associated with important clinical impact in terms of safety and efficacy. The aim of our study was to assess if the origin of the biological drug (murine, humanized or human) affects antibody development.

Blood samples from 484 patients treated with these immunomodulatory drugs at 12 Octubre Hospital were collected during 29 months between 2016 and 2018. All serum samples were evaluated for drug levels and those that resulted in undetectable drug levels were evaluated for the presence of non-isotype specific anti-drug antibodies using a commercially available bridging format ELISA kit (Promonitor®, Proenika Biopharma S.A., Spain), according to the manufacturer’s instructions. Twenty three out of 257 patients with murine monoclonal antibody infliximab (8.95 %), 21 out of 207 (10.14%) patients with human monoclonal antibody adalimumab and any patient with human recombinant protein etanercept developed anti-drug antibodies. The anti-drug antibodies presence didn’t seem to be significantly different between infliximab (murine) and adalimumab (human). Anti-drug antibodies triggers negatively influences to the treatment response so it is important to determine the factors that can promote its development. In this study, we demonstrate that the origin of immunomodulatory drugs do not affect the later anti-drug antibody production. Owing to these monoclonal antibodies are produced in the same cell line, post-translational modifications could be the shared immunogenic cause.

P.C3.01 Bone Marrow Transplantation

P.C3.01.01

The glucocorticoid receptor in recipient cells keeps cytokine secretion in acute graft-versus-host disease at bay

T. K. Baade1,2

1Institute for Cellular and Molecular Immunology, Georg-August-University, University Medical Center, Göttingen, Germany.

Introduction: Acute graft-versus-host disease (aGVHD) is a life-threatening complication of hematopoietic stem cell transplantation (HSCT) and considered as the main risk factor for transplant-related morbidity and mortality. First-line therapy of GVHD is usually accomplished by glucocorticoid (GC) application but the mechanisms and target cells of this treatment regimen are not yet fully understood. Objectives: Here we analyzed the role of the glucocorticoid receptor (GR) in recipient myeloid cells and determined how its deletion in mice influences mortality, clinical symptoms, intestinal tissue damage as well as local and systemic cytokine production after allogeneic HSCT. Materials & methods: A total of 240 MHC-mismatched model of allogeneic HSCT were used to induce aGVHD in GR−/− mice lacking the GR in myeloid cells. Bone marrow and purified T cells were isolated from wildtype C57Bl/6 mice and transferred into irradiated Gr−/− and Gr−/−BALB/c mice. The disease course was followed over a period of 6 weeks, histological and immunological analyses were performed on day 4, 6 and 8 post-transplant. Results: Deletion of the GR in recipient myeloid cells strongly exacerbates aGVHD, an effect which is unrelated to local GC effects in GvHD target organs but rather caused by uncontrolled systemic cytokine release. Conclusion: The regulation of recipient myeloid cells by GC is essential to prevent a lethal cytokine storms after allogeneic HSCT. Selectively targeting this cell type might therefore offer a new strategy to treat aGVHD patients.

P.C3.01.02

Outcome of mesenchymal stromal cell treatment in chronic GVHD predicted by thymic function: A phase II clinical study

E. Boberg1,2, L. von Bahr1, N. Heldring3, G. Afram1, E. Alici1, E. Iacobaeus1, K. Garming Leger1, P. Petzelbauer1, P. Ljungman1, R. Sugars2, N. Kadri1, K. Le Blanc1

1Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, 2Center for Hematology and Regenerative Medicine, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden, 3Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska University Hospital, Stockholm, Sweden, 4Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden, 5Skin & Endothelial research division SEDR, Department of Dermatology, Medical University of Vienna, Vienna, Austria, 6Division of Hematology, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

Introduction: Treatment options are limited for chronic graft-versus-host-disease (cGVHD) refractory to thymic treatment. Isolation of mesenchymal stromal cells (MSCs) is a promising alternative but the mechanism of action has not been established.

Methods: 11 patients with severe steroid refractory cGVHD were treated with repeated infusions of allogeneic bone marrow MSCs. Clinical response was evaluated using the National Institutes of Health (NIH) criteria. Immunophenotyping and cytokine analysis was conducted on blood samples taken before, and at several time points shortly after each infusion. Skin biopsies were taken before and after treatment.

Results: Nine patients completed the treatment protocol and were evaluated for response. Six patients responded to MSC therapy according to NIH criteria. The responders had significantly higher proportions of naïve Th-cells (CD3+CD4+CD45RA+CD27+) and activated naïve B cells (CD19+gDcCD38low). The proportion of Ki67+ naïve Th-cells was similar in both responders and non-responders, while the percentage of recent thymic emigrants naïve CD4+ T-cells were higher in responders, suggesting a better thymic function. Several miRNAs increased one hour after MSC infusion in all patients, followed by transient increases in absolute numbers of naïve and regulatory T- and B-cells after 7 days in responders. Long-term reductions were seen in the cytokines CXCL2 and CCL2 in responders, while the levels of CXCL9, CXCL10, CXCL12, TNFα and IL-6 increased in non-responders. Skin biopsies showed resolution of epidermal pathology.

Conclusion: Our data highlights the importance of the recipient immune phenotype for response to MSC treatment of cGVHD and suggests a link to thymic function.

P.C3.01.03

In silico analysis of allotriantigen landscape in HLA-matched transplantation

N. A. Bykova1, D. B. Malia1, G. A. Efimov1

1National Research Center for Hematology, Moscow, Russia Federal.

Minor histocompatibility antigens (MIHAs) are allotriantgens in the context of HLA-matched allogeneic hematopoietic stem cells transplantation (allo-HSCT). Due to the crucial role they play in graft versus leukemia effect MIHAs were suggested as possible targets for immunotherapy. However, the restricted number of clinically relevant MIHAs known so far limits their applicability. To make anti-MHA therapy broadly usable systematic discovery of novel MIHAs is needed. In the current study we performed in silico analysis of the genomic data to describe basic features of MIHA landscape. For this, pairs of samples were arbitrary selected from 1000 Genome project, as donor and recipient. Unique peptides found in the recipient genotype that were predicted to bind MHC molecules with high affinity were considered as potential MIHAs.
We further compare the results with known and mass spectrometry (MS) predicted MiHAs. The analysis demonstrated that at the moment our knowledge about MiHAs is largely incomplete. MS approaches to predict known MiHAs, probably missing significant amount of potentially relevant MiHAs; therefore the co-dominant/ dominant MiHAs is inconsistent between in silico predicted and MS predicted or known MiHAs; MS predicted peptides are biased towards high allele frequency of immunogenic allele, which is suboptimal for usage in immunotherapy. Lower estimations of in silico approach suggest that there are at least several dozen strong MHC-binding SNP-associated alleles in the allele frequency region optimal for off-the-shelf therapeutics, that are not still experimentally tested for immunogenicity. Funding was provided by Russian Science Foundation grant 17-15-05112.

P.C3.01.01
Discovery of HLA class I-restricted minor histocompatibility antigens by a new approach for whole genome association scanning


Minor histocompatibility antigens are polymorphic peptides presented on the cell surface by HLA molecules. After allogeneic stem cell transplantation (SCT) as treatment for hematological malignancies, genetic differences in single nucleotide polymorphisms (SNPs) between patient and donor result in presentation of minor histocompatibility antigens that are recognized by donor T-cells. These T-cells can induce the favourable Graft-versus-Leukemia effect, but also mediate undesired Graft-versus-Host Disease. In our laboratory, we have previously discovered an SNP-based humanised mouse model that has been developed by transgenic strategy. In this mouse model, donor T-cells isolated from patients after allogeneic SCT are tested for recognition of a panel of 80 B-lymphoblastoid cell lines, and SNPs that strongly associate with T-cell recognition are subsequently validated to encode the antigens. The number of SNPs that were measured for this panel, however, was limited to one million. Utilizing a new panel of 191 B cell lines, which are sequenced in the 1000 Genome Project, we now optimized the approach and increased SNP coverage to 12 million. Furthermore, while our previous panel was restricted to few HLA, the new panel includes six common HLA class I alleles. Currently, genome association scanning has been successfully applied and various minor histocompatibility antigens have been found. The aim is to use the optimized method to identify the dominant repertoire of HLA class I-restricted minor histocompatibility antigens, since knowledge about mismatched antigens may allow for a personalized treatment after transplantation as well as a more directed donor selection thereby contributing to a better outcome of patients treated with allogeneic SCT.

P.C3.01.05
The impact of stem cell graft γδ T cells on clinical outcome after allogeneic hematopoietic stem cell transplantation

A. Gaballa, A. Stilkevicius, B. Onfer et al.; 1Karolinska Institutet, Stockholm, Sweden, 2Royal Institute of Technology, Stockholm, Sweden.

The impact of donor graft composition new kidneys partly introduces unknown HLA, probably missing significant amount of potentially relevant MiHAs; the ratio of co-dominant/ dominant MiHAs is inconsistent between in silico predicted and MS predicted or known MiHAs; MS predicted peptides are biased towards high allele frequency of immunogenic allele, which is suboptimal for usage in immunotherapy. Lower estimations of in silico approach suggest that there are at least several dozen strong MHC-binding SNP-associated alleles in the allele frequency region optimal for off-the-shelf therapeutics, that are not still experimentally tested for immunogenicity. Funding was provided by Russian Science Foundation grant 17-15-05112.

P.C3.01.07
The CD73/A2A signalling axis impacts graft-versus-host disease in a humanised mouse model

N. J. Geraghty, S. R. Adhikary, D. Watson, R. Sluyter; University of Wollongong, Wollongong, Australia.

Introduction: Graft-versus-host disease (GVHD) is a major cause of mortality following allogeneic hematopoietic stem cell transplantation. GVHD occurs when T cells in the graft cause inflammatory damage to host tissues. Ecto-5'-nucleotidase (CD73) generates extracellular adenosine, which activates the adenosine 2A (A2A) receptor to limit T cell responses. In allogeneic mouse models of GVHD, CD73 or A2A blockade worsens, whilst A2A activation limits disease. The current study examined the role of the CD73/A2A signalling axis in a humanised mouse model of GVHD. Material and Methods: NOD-SCID-IL2γ−/− (NSG) mice, injected i.p. with 10×10^6 human (h) peripheral blood mononuclear cells, were subsequently injected i.p. with h-methyleneEDP (CD73 antagonist) or GS251680 (A2A agonist) for 6 or 14 days, respectively. GVHD development was assessed by weight loss, clinical parameters, survival and histology. Immune cell markers and cytokines were analysed by flow cytometry. Results: Neither CD73 blockade or A2A activation impacted initial human leukocyte engraftment or survival. CD73 blockade worsened GVHD as evidenced by increased weight loss, liver histology and serum hIL-2. Contrary to expectations, A2A activation increased weight loss, decreased human regulatory T cells and increased serum hIL-6. A2A activation however decreased serum hTNF-α and hIL-2, but did not alter histology of target tissues. Conclusion: CD73 blockade or A2A activation reduces the health of mice in this humanised model of GVHD. The CD73 blockade effects may be explained by accumulation of proinflammatory ATP rather than reduced adenosine production. The effects of A2A activation may reflect reduced metabolism or food intake rather than GVHD per se.

P.C3.01.08
The fast NKG2C+ NK “memory” expansion in CMV reactivation patients post allogeneic Stem Cell Transplantation

P. Moss, J. Nunnick, P. Moss et al.; 1Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom, 2Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom, 3Birmingham Health Partners, Centre for Clinical Haematology, Queen Elizabeth Hospital, Birmingham, United Kingdom.

Introduction: Human haemopoietic stem cell transplantation (H SCT) is the main treatment option for patients with severe hematological diseases. Mucositis/macrophage (MCM) reactivation in allogeneic haematopoietic stem cell transplantation (H SCT), regularly leads to HCMV disease and can cause serious morbidity and mortality. The relation between CMV-reactivation and leukemia recurrence in patients post HSCT is highly controversial and there is increasing evidence to show that CMV-reactivation is associated with the decrease rate of Leukemia relapse post HSCT. Methods: The phenotypic of NK cell in the peripheral blood mononuclear cells (PBMCs) in H SCT patients with HCMV-reactivation was studied using flow cytometry. The HSCT patients without HCMV-reactivation and healthy donors PBMCs were used as a control. The markers analysed for NK phenotypes include CD56, CD16, CD94/NKG2C, CD94/NKG2A, KIRs, CD57, KIRLG1 and PD-1. Results: NK cell surface expression markers were compared between HSCT patients with HCMV-reactivation and without HCMV-reactivation. The NK cells from both patient groups have similar composition of CD56+/CD16−/− and CD56+/CD16+. Strikingly, the percentage of CD56+/16+/100%NKG2C+ population in HCMV-reactivation patients accumulated overtime when compared with no reactivation. Before HSCT, there are 6.4±3.5% (Mean±SEM) NK2C+ NK cells in HCMV-reactivation group compared with 2.9±1.4% NKG2C+ NK cells in non-reactivation group. 10 months later, there are significantly higher NKG2C+ NK cells in HCMV reactivation group (35.8±10.4% versus 8.8±2.9%) overtime when compared with no reactivation. Before HSCT, there are 6.4±3.5% (Mean±SEM) NKG2C+ NK cells in HCMV-reactivation group comparing with 2.9±1.4% NKG2C+ NK cells in non-reactivation group. 10 months later, there are significantly higher NKG2C+ NK cells in HCMV reactivation group (35.8±10.4% versus 8.8±2.9%). Conclusions: This study has shown the fast accumulation of CD56+/16+/100%NKG2C+ NK population in HSCT patients with HCMV-reactivation. We hypothesise that NK population will contribute to the reduction of Leukaemia relapse in patients with HCMV-reactivation. This study will help to guide the future management of HCMV-reactivation and develop strategies to reduce disease relapse.

P.C3.01.09
Exploring immunological mechanisms of human graft-versus-host-disease after hematopoietic stem cell transplantation

E.Letts,1 C. Leloup,1 D. Michonneau,1 A. Garcia,1 E. Bianchi,3 G. Socié,1 A. E. Adriaans, M. W. Honders, E. D. van der Meijden, M. J. Pont, R. Monajemi, S. M. Kielbasa, P. A. ‘t Hoen, C. A. van Bergen, J. Falkenburg, M. Griffioen; 1Institut Pasteur, Immune Regulation Unit, Department of Immunology, Paris, France, 2Université Paris Diderot, Paris, France, 3Service Hematologie Greffe, Hôpital St Louis, Paris, France.

Allogeneic hematopoietic stem cell transplantation (allo-HCT) is a curative treatment for many hematologic malignancies. However, its success is hindered by graft-versus-host disease (GVHD), a life-threatening complication deriving from alloreactive donor T-cells attacking recipient (host) tissues. Despite the advances in the field of HCT and GVHD prophylaxis, disease processes in humans remain poorly understood, and the lack of biomarkers for the early diagnosis and prognosis contributes to the high mortality of the disease. In this study we investigated the mechanisms involved in immune reconstitution early after transplantation and in acute GVHD onset. For two independent cohorts of related HLA-identical donor-recipient couples, blood was collected either at GVHD onset, before any treatment, or at day 90 post-HSCT for recipients not developing GVHD. We have performed molecular profiling of cell populations important for GVHD development as well as immune-phenotyping using spectral flow cytometry. Molecular profiling showed that donor T-cells react to the environment of the host by acquiring an activated phenotype, upregulating genes associated with T-cell activation, co-stimulation, migration and effector functions. Cellular profiling showed an incomplete reconstitution of the T-cell compartment in recipients early after transplantation, and a depletion of naive T-cells associated with an increase of cells with an effector-memory phenotype after HSCT. Finally, we noted an increase of cells with a T memory stem cell-like (TSCM-like) phenotype at GVHD onset. These cells may represent a cellular reservoir for GVHD maintaining the production of alloreactive T cells in the presence of host persistent antigens.

P.C3.02.02
The role of ATP receptor A2A in Fibrosis and Graft-versus-Host Disease (GVHD) in a Humanized Mouse Model

1Université Paris Diderot, Paris, France, 2Univeristé Paris Diderot, Paris, France, 3Service Hematologie Greffe, Hôpital St Louis, Paris, France.
Poster Presentations

PC.C.01.10

Allograft of host macrophages exacerbates acute GvHD

D. Le1, M. Raneczyk1, A. Jordan Garrote1, K. Ottmüller1, H. Shaikh1, M. Qureschi1, L. Scheller1, T. Steinfatt1, A. Brandl1, Z. Mokhtar1, J. Delgado Tascón1, J. Hartweg1, A. Mottok1, H. Eisene1, E. Vafadarenejad1, A. Solís1, M. B. Lutz1, A. Beilhack2

1Department of Medicine II, Wuerzburg, Germany; 2Institute of Pathology, Wuerzburg, Germany, 3Helmholtz Institute for RNA-based Infectious Research (HIRI), Wuerzburg, Germany, 4Institute for Virology and Immunobiology, Wuerzburg, Germany.

Allogeneic hematopoietic cell transplantation (allo-HCT) can lead to the severe complication of intestinal acute graft-versus-host disease (aGVHD). Host tissue resident antigen-presenting cells are considered as essential components in aGVHD pathogenesis. However, in recent years it has become clear that hematopoietic host APCs are not absolutely required to initiate acute GvHD and that non-hematopoietic host APCs are sufficient to effectively activate alloreactive T cells to trigger acute GvHD. Therefore, the role of hematopoietic APCs in GvHD has not yet been clearly defined.

Here we report that depletion of host APCs, which highly express CD11c, leads to exacerbation of GvHD as seen from reduced survival and increased clinical score after allo-HCT. Using reporter mice for dendritic cells (Zbta46-GFP), novel macrophage marker panels for flow cytometry (MHCIi, CD11c, F4/80, CD64, CD206) and single cell sequencing we show for the first time that the intestinal CD11c-expressing myeloid cells, which survived after irradiation, are macrophages but not dendritic cells. Moreover, our results indicate, that host macrophages can reduce intestinal aGVHD via PD-L1.

Our data indicate a protective role of host macrophages in aGVHD and suggest that targeting intestinal host macrophages may represent a novel therapeutic strategy for aGVHD.

PC.C.01.11

Predictive Surface Biomarkers for Murine Acute Graft-versus-Host Disease

M. Qureschi1, C. Bäuerlein1, C. Brede2, A. Jordan Garrote2, S. Riedel2, M. Chapra3, A. Mottok2, S. Thusek2, M. Ritz2, K. Mattenheimer4, C. Graf2, H. Eisene1, R. Negroni5, P. Schlegel6, A. Beilhack2

1Centre for Experimental Molecular Medicine, Würzburg, Germany, 2Division of Blood and Marrow Transplantation, Stanford University, Germany; 3Department of Pediatrics, Würzburg, Germany.

Acute graft-versus-host disease (aGVHD) is a severe inflammatory complication of hematopoietic cell transplantation. aGVHD is mediated by donor T cells attacking the gastrointestinal tract, liver, and skin, Efficient strategies to improve aGVHD-related morbidity and mortality will rely on more precise methods to predict aGVHD and abrogate disease manifestation. Here, we asked whether the combination of surface receptors, particularly chemokine receptors and adhesion molecules, would identify alloreactive donor T cells before the onset of acute GvHD. To this end we employed multiparameter flow cytometry in two independent murine allo-HCT models to address whether defined markers alone or in combination could predict the onset of GvHD. C57BL/6 (H-2b, Thy1.1+) or BALB/c (H-2d, Thy1.2+) T cells plus bone marrow cells were transplanted in conditioned (B6g) male or female congenic recipient mice (Thy1.2+ or BALB/B [H-2d, Thy1.1+] recipients). To identify suitable predictive markers, we compared the expression pattern of allo-HCT recipients to syngeneic HCT recipients and untreated controls. Notably, the chemokine receptor expression (CCR2, CCR4, CCR5, CCR6, CCR7, CCR9) did not differ in the PB of allologenic vs syngeneically transplanted mice. However, we found an upregulation of sILP integrin, and E- and P-selectin ligand on allologenic PB T cells early after allo-HCT.

The combination of these homing markers with the activation markers CD25 and CD69 at later time points and low expression levels of L-selectin allowed to differentiate alloreactive donor T cells. Based on these data, we propose a potential predictive blood test, which could allow a timely therapeutic intervention before clinical aGVHD manifestation.

PC.C.01.12

Serine protease activated Protein C alters T cell plasticity in GvHD by inducing anergy and metabolic quiescence in Teffector cells

S. Ranjan1, D. Gupta1, A. Goel1, B. Schraeven1, B. Iseman1

1Institute of Chemical Pathology and Pathobiology, Magdeburg, Germany; 2Institute for Molecular and Clinical Immunology, Magdeburg, Germany.

Introduction: The serine protease activated protein C (aPC) is an anticoagulant protease. The role of aPC in controlling innate immunity through both its anticoagulant and signaling properties is well recognized. We have recently demonstrated a novel function of aPC and its receptors (protease activated receptors) in adaptive immunity and shown that aPC ameliorates GvHD. Here we test the hypothesis that aPC alters T cell plasticity in GvHD. Material and Methods: In vitro proliferation assays were done by mixed lymphocyte reactions with aPC and its receptor. aPC is able to induce anergy and metabolic quiescence (reduced glycolysis and mitochondrial respiration) in Teff cells after 24h of stimulation. In vivo aPC also reduced phosphorylation of Akt, mTORC1 and p70S6 kinase in human T cells. In vivo aPC improves GvHD in mice by inducing anergy and altering T cell plasticity. This effect is associated with improved survival, lower clinical scores, and lesser tissue damage and apoptosis in the gut. Conclusions: This data demonstrate that aPC induces anergy and metabolic quiescence and aPC ameliorates experimental GvHD in mice by altering T cell plasticity.

PC.C.01.13

In vitro-generated Th9 cells mediate anti-tumor cytotoxicity against B cell lymphomas in the absence of graft-versus-host disease (GVHD)

T. Reisser1, I. Knape1, I. Scheurer1, F. Lethäuser1, K. Debattin1, G. Strauss2

1University Medical Center and Department of Pediatrics and Adolescent Medicine, Ulm, Germany; 2Institute of Pathology, University of Ulm, Ulm, Germany.

Introduction: T helper 9 cells (Th9) are characterized by the massive production of IL-9 and contribute to immunopathogenesis in autoimmune disease, but mediate immune responses against hemorrhage induced solid tumors. GvHD is the most frequent and severe complication after allogeneic bone marrow transplantation (BMT) induced by mature donor-derived T cells destroying recipient target organs.

Methods: We explored in different MHC-mismatched BMT models, whether in vitro-generated Th9 cells influence GVHD development and whether they exhibit a graft-versus-tumor (GVT) effect. Therefore, alloimmune in vitro generated Th9 cells were co-implanted with bone marrow cells and different hematological tumor cell lines.

Results: 5 days of Th9 differentiation from naive CD4 T cells with TGF-β, IL-4, anti-IFN-γ and TL1A achieves >60% IL-9+ TNF-α+ IFN-γ- cells distinguishable by their cytokine profile from other Th subsets. In different BMT models, in vitro-generated Th9 cells did not induce clinical and histological GVHD or systemically increase the GvHD-associated cytokines TNF-α and IFN-γ. Th9 cells migrated to but did not attack GVHD target organs, but changed their phenotype into IL-9+ TNF-α+ IFN-γ- producing cells. Regarding the GVT effect, Th9 cells could efficiently prevent tumor development of B cell lymphomas but did not exhibit anti-tumorigenicity against mastocytomas or thymomas.

Conclusion: Adaptively transferred Th9 cells elicit effective anti-tumorigenicity against different B cell lymphomas while other tumor entities are not recognized. Th9 cells might therefore have the potential to serve as therapy in BMTs to eliminate residual tumor cells. Future experiments will elucidate the molecular mechanism of Th9 selectivity towards B cell malignancies.

PC.C.01.14

Minor histocompatibility antigens as cell therapy target and GvHD predictor after allogeneic hematopoietic stem cell transplantation

D. Romanuik1, A. Pustowskaya2, A. Kmenievskaya2, N. Bykova1, D. Malko1, G. Efimov1

1National Research Centre for Hematology, Moscow, Russian Federation; 2Institute for Molecular and Clinical Immunology, Magdeburg, Germany.

Use of fully HLA-matched donor for HCT does not completely prevent development of graft versus host disease (GvHD), which could be driven by minor histocompatibility antigens (MiHA). MiHAs are polymorphic peptides, presented in context of HLA. When MiHA coding gene expression is restricted to the hematopoietic lineage a mismatch could lead to selective graft-versus-leukemia (GvL) reactivity, lowering the risk of relapse. MiHA genotyping of donor-patient pairs is a promising approach to predict severe side effects and to select targets for immunotherapy. We designed multiplex aPCR MiHA genotyping method, and genotyped 25 pairs (10 siblings) for 20 antigens presented in HLA-A*02,01. Related and unrelated pairs have 1-5 (median 2) and 2-7 (median 4) mismatches (MiM) respectively. Using expression data from Human Protein Atlas we separated MiHA-coding genes to identify suitable predictive markers, we compared the expression pattern of allo-HCT recipients to syngeneic HCT recipients and untreated controls. Notably, the chemokine receptor expression (CCR2, CCR4, CCR5, CCR6, CCR7, CCR9) did not differ in the PB of allologenic vs syngeneically transplanted mice. However, we found an upregulation of sILP integrin, and E- and P-selectin ligand on allologenic PB T cells early after allo-HCT.

The combination of these homing markers with the activation markers CD25 and CD69 at later time points and low expression levels of L-selectin allowed to differentiate alloreactive donor T cells. Based on these data, we propose a potential predictive blood test, which could allow a timely therapeutic intervention before clinical aGVHD manifestation.
Second malignancies are a well-defined late complication after hematopoietic stem cell transplantation (HSCT) which includes myelodysplastic syndrome (MDS) and acute myeloid leukemia developed de novo in engrafted cells of donor origin. These donor cell-derived myelodysplastic syndrome (DCM) and donor cell-derived leukemia (DCL) are intriguing phenomena which are considered to carry an extremely poor prognosis, consistent with other secondary leukemias. Intuitively, a different mismatched donor would seem the best therapeutic option since significant graft-versus-leukemia effect would not be anticipated in the total absence of HLA disparity between donor T cells and the leukemic clone. This case report describes a 62 years old man diagnosed of Philadelphia chromosome positive chronic myeloid leukemia in chronic phase which relapsed after 20 years from receiving an allogenic HLA-identical sibling HSCT transplantation while maintaining a complete donor chimerism. The patient developed a DCM and a donor-cell monochromatic gamopathy of undetermined significance (MCLS), which finally progressed to a myelodysplastic and smoldering multiple myeloma, respectively. Interestingly, in the meantime his donor also developed a MDS and an MGS with the same paraprotein isotype as the recipient, which support the existence of a primary inherited gene defect constituting one of the very first hits in the pathogenesis of blood cancers. The patient was successfully treated with HSCT from his son donor and remains in complete remission to the date after 33 months, which supports the hypothesis that a haploidentical HSCT is the more logical consolidation strategy in donor cell-derived haematological malignancies.

POSTER PRESENTATIONS

P.C3.01.15 Long-term complete remission after haploididentical allogeneic hematopoietic stem cell transplantation in a 62-year-old man with donor cell myeloid leukemia and multiple myeloma after allogeneic hematopoietic stem cell transplantation for the treatment of chronic myeloid leukemia

S. Sánchez Alonso, A. Alcoraz, J. L. Steegman, A. Figuera, A. Alegre, C. Muñoz; Hospital Universitario de La Princesa, Madrid, Spain.

P.C3.01.16 Rapamycin increases myeloid-derived suppressor cells (MDSC) activation and immunosuppressive function in protecting against graft-versus-host disease (GVHD) in a murine allogeneic bone marrow transplantation (BMT) model

J. Scheurer1, T. Reisser1, J. Knape1, F. Leithäuser1, K. Holzmann1, K. Debati1, G. Strauss1.
1Department of Pediatrics and Adolescent Medicine, 89075 Ulm, Germany, 2Institute of Pathology, 89075 Ulm, Germany, 3Genomic-Care Facility, 89075 Ulm, Germany.

Introduction and Objectives: MDSCs are a heterogeneous population of myeloid progenitors, which suppress T-cell functions and expand during inflammation. GVHD also induces MDSCs, which however don’t protect from GVHD-induced death. Rapamycin is a macrodilute immunosuppressant with protective effects against GVHD. The effect of Rapamycin on MDSC population and GVHD regulation isn’t well defined. We explored the effect of Rapamycin in GVHD prevention, (2) on MDSC-induction, activation and immunosuppressive function and (3) on changes in T-cell function, polarization and exhaustion. Methods: Allogeneic bone marrow transplanted mice were treated with Rapamycin or PBS every 2nd day. MDSCs were isolated from spleens and GVHD target organs for defining transcriptional changes and their immunosuppressive capacity. Allogeneic T-cells were analyzed for changes in the expression of cytokines, cytotoxic molecules and exhaustion markers. Results: Rapamycin treatment effectively prevented clinical and histological GVHD and increased survival. Rapamycin increased splenic MDSC numbers and enhanced their suppressive potential towards alloreactive T-cells. Rapamycin-activated MDSCs significantly up-regulated NOS expression, while immunosuppressive factors arginase-1, HO-1, CD25, and TGF-β remained unchanged. Transcriptomic analysis revealed distinct transcriptional changes in Rapamycin-activated MDSCs, most evident for genes involved in innate and inflammatory responses, and in the regulation of secretion. Despite Rapamycin induced MDSC-activation, no alterations in T-cell exhaustion, Th1/Th2 polarization or expression of cytotoxic molecules were detectable. Conclusion: Rapamycin is an effective adjuvant for GVHD prevention, which induces and activates highly immunosuppressive MDSCs. Further experiments will clarify, whether Rapamycin treatment maintains the graft-versus-tumor effect, major in allogeneic BMT therapy.

P.C3.01.17 Non-hematopoietic Antigen Presenting cells in lymphoid organs

H. Shaikh1, M. Queirisch2, J. Otmüller1, M. Otto, D. Le1, A. Beilhack1.
1Department of Internal Medicine II, University Hospital Würzburg, Würzburg, Germany, 2Graduate School of Life Sciences, Julius-Maximilians University Würzburg, 97078 Würzburg, Germany.

Hematopoietic stem cell transplantation is a curable therapy for hematopoietic malignancies. Allogeneic T-cells residing within the graft have potential to eliminate remaining malignnant cells by Graft versus leukemia effect. Nevertheless, in 30-60% of HSCT cases allogeneic T-cells become alloreactive and attack host tissues resulting in acute Graft versus Host Disease. Dendritic cells are professional antigen presenting cells (APCs) and thought to be the prime candidate for presenting self-antigens to allogeneic T-cells. However, recent findings claim that even in absence of DCs and other hematopoietic APCs GVHD cannot be prevented, suggesting role of non-hematopoietic APCs in the activation of allogeneic T-cells. In this project, we investigate role of lymph node stromal cells (LNStCs) in initiation phase of aGVHD and their potential role as non-hematopoietic APCs.

We show that T-cells are activated in lymphoid organs and not in GVHD target organs. In MHC-II deficient bone marrow chimeras bearing MHC-II non-hematopoietic APCs, allogeneic T-cells still get activated but to a lesser extent. Furthermore, LNStCs upregulate co-stimulatory receptors early after conditioning suggesting their contribution as active antigen presentation cells in initiation phase of aGVHD. However, LNStCs significantly downregulate MHC-II on their surface as LNStCs have been shown to acquire MHC-II from higher than for LB-NDC80-1P(f≈2*107) and ACC-1Y(f≈3.5*107) antigens, which corresponds to their immunogenicity in vivo. This data can be useful for selection of the donor and immunosuppressive regimen and for the graft design. Funding was provided by Russian Science Foundation grant 17-15-0152.

P.C3.01.18 Unraveling the minor histocompatibility antigen immunogenicity through naïve T cell fate extensions

1National Research Center for Biotechnology, Moscow, Russian Federation, 2Ghent University, Ghent, Belgium.

Allogeneic bone marrow transplantation is the potentially curative treatment for malignant hematopoietic disease. The therapeutic effect depends on the immune response of donor lymphocytes to residual tumor cells (graft-versus-leukemia effect). At the meantime broader immune response of transplant towards recipient could be detrimental (graft-versus-host disease). In HLA-matched transplantation donor’s lymphocytes recognize the differences in peptides repertoires displayed within MHC as foreign antigens (minor histocompatibility antigens). The magnitude of immune responses determines the development of graft-versus-host disease or graft-versus-leukemia reaction. Different minor histocompatibility antigens differ in their immunogenicity in vivo. Our hypothesis is that this can be explained by the unequal frequency of naive antigen specific T cells in the donor repertoire. In the current study we examined the naïve T cell pool specific for antigens HA-1, HA-2, ACC-1Y, LB-ADIR-1F and LB-NDC80-1P. Donors were genotyped for minor antigens and HLA, and selected on the basis of having restricting HLA allele and lacking allele encoding the minor antigen of interest. By culturing naïve T cell with dendritic cells loaded with minor antigen peptides we expanded and detected rare antigen specific clones and estimated their frequencies with limiting dilution approach. Our results show that the incidence of naive precursor specific for the LB-ADIR-1F(a≈3.5*105) and LB-NDC80-1P(a≈2*105) antigens is significant higher, than for LB-NDC80-1P(a=2*104) and ACC-1Y(f≈2*104) antigens, which corresponds to their immunogenicity in vivo. This data can be useful for selection of the donor and immunosuppressive regimen and for the graft design. Funding was provided by Russian Science Foundation grant 17-15-0152.

P.C3.01.19 Expression of CD38 is elevated on peripheral blood lymphocytes of patients that do not develop chronic GVHD after HSCT

D. Tomaz1, R. Ellis1, N. Petrov1, S. Heck2, G. J. Mufti1, V. Mehra2, S. Kordasti3, L. D. Barber3.
1School of Cancer and Pharmaceutical Sciences, King’s College London, London, United Kingdom, 2National Institute of Health Research Biomedical Research Centre, Guy’s Hospital, London, United Kingdom, 3Department of Paediatric Hematology, King’s College Hospital, London, United Kingdom.

Chronic graft-versus-host disease (cGVHD) is a significant cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (allo-HSCT). CD38 is associated with immunosuppressive activity, being often increased in cGVHD patients. In this study, we performed a comprehensive donor versus recipient clonotypic mass cytometry analysis of peripheral blood lymphocytes from nine patients with cGVHD and eight patients without cGVHD after allo-HSCT using allemutumbab for lympho-depletion, and eight healthy volunteers. PhenoGram Clustering tools were used for data analysis. Patients with cGVHD were found to have a paradoxically higher frequency of CD4+ T cells compared to those without cGVHD (p=0.018). CD4+ T cells had a higher frequency of exhausted phenotype (FoxP3low, CD45RAnegative) CD4+ Treg cells compared to patients without cGVHD (p=0.028), who had a higher frequency of activated phenotype (FoxP3high, CD45RAnegative). CD4+ Treg cells (p=0.032). Notably, we observed that the frequency of CD38+ Treg cells and expression levels of this marker were significantly elevated in patients without cGVHD compared to those with active cGVHD. Moreover, expression of CD38 was also significantly elevated in CD4+ T cells, CD8+ T cells and NK cells in patients without cGVHD. In conclusion, the activated CD4+ Treg phenotype and comparatively higher expression of CD38 on lymphocytes in allo-HSCT patients without cGVHD suggests an increased ability to modulate allo-immune responses.
POSTER PRESENTATIONS

P.C3.01.20
Antigen-experienced subsets of cytomegalovirus-specific T-cells substantially differ in clonality
M. Vegido, N. Bykova, G. Efimov;
National Research Center for Hematology, Moscow, Russian Federation.
Cytomegalovirus infection is a serious complication after allogeneic hematopoietic stem cell transplantation. One of the promising therapeutic strategies is adoptive transfer of virus-specific T-lymphocytes. Central memory cells have more proliferative potential than other memory subsets and might be more applicable for therapy. In this work, we sorted three populations of CD8+ lymphocytes, specific for HLA-A02 immunodominant cytomegalovirus epitope - NLV: effector memory, terminal effectors and central memory. RNA was isolated from sorted cells as a template for cDNA synthesis and was used as adapters attaching to each cDNA molecule. DNA library was sequenced on the Illumina platform. Studied T-cell subpopulations drastically vary in the diversity of T-cell receptors. The ratio of unique clonotypes to total cells was much higher for central memory T-cells 0.349 than 0.127 and 0.022 for effector memory and terminal effectors, respectively. Terminal effector repertoire is extremely contracted, compared with repertoires from two other subpopulations. This effect could be a result of proliferation only of small numbers of clones during differentiation to terminal effector T-cells. The high number of unique clonal distributions of memory fraction may be the result of a smaller niche and the absence of expansion in the process of differentiation from naive cells. The difference in diversity of T-cell receptor repertoires of CD8+ virus-specific T-cell subsets may result in different therapeutic potential of these cells. This work was supported by the Russian Foundation for Basic Research grant 17-04-00888.

P.C3.02.01
Regulation of perforin secretion and degranulation by TCR stimulation in human NK cells
S. Betriu Méndez, J. Rivora*, B. Morral*, E. Bonan-Manousse, A. Mulder, F. Clas, M. Juan, F. Diekemmer, E. Pauleu*
1Fundació Clinic, Hospital Clinic de Barcelona, Barcelona, Spain, 2Academic Hospital Leiden, Leiden, Netherlands.
Introduction: Chimeric antigen receptor (CAR) T cell therapy has emerged as a promising approach to combating cancer with excellent results in anti-leukemic clinical trials, and a broader appraisal, promising immunotherapeutic results. A major problem in solid organ transplantation is the presence and the generation of de novo in the recipient of donor specific antibodies which preclude the success of the transplant due to the associated high risk of antibody-mediated rejection. B cells contribute in the acute and chronic alloimmune reaction with the production of anti-HLA antibodies. We hypothesize that a CAR (chimeric antigen receptor)-like molecule with a particular A2M molecule as the CAR extracellular domain will engineer T cells to kill alloimmune B cells with anti-HLA antibodies as B cells completely eliminating alloantigens in a specific manner.
Methodology and Results: First step, we cloned the extracellular region of HLA-A*02:01 from A*02:01 positive donor cDNA by PCR. We constructed CARs with this extracellular domain and 4-1BB/CD3ζ intracellular signalling domains and delivered into human T cells by lentiviral transduction. Next, to evaluate the specificity and functionality of CAR T cells, we performed a cytotoxicity assay by co-culturing them with anti-HLA A2 alloantigen presenting B cells. Conclusion: HLA CAR-like receptor has been constructed in our laboratory and used to generate specific T cells directed to kill anti-HLA alloantigen presenting B cells. This technology could open new ways of treatment and prevention of antibody-mediated rejection in solid organ transplantation.

P.C3.02.02
Reconstituted allogenic immune cells direct resistance to tumors in mice
N. Dang, Y. Lin, O. Rudgeerts, A. D. Billiau, M. Waer, B. Sprangers;
KU Leuven, Leuven, Belgium.
Abstract: acute graft-versus-host disease (aGVHD) is an often-lethal syndrome resulting from immune reconstitution and cytotoxic T cells (CTLs) response to allogeneic bone marrow transplantation (BMT). Regulatory T cell (Treg) mediated-tolerance is a defense strategy against aGVHD that has a direct positive impact on resistance to tumor - graft-versus-tumor (GVT) effect. Here, we demonstrate that induction of Treg cells in response to tumor milieu is critical to establish tolerance to aGVHD after T cell-replete BMT. The protective effect of donor-derived Treg cells is exerted via a mechanism that counteracts Teff/Treg ratio, and in doing so, M2 polarization as a result of relatively limited tumor-associated macrophages (TAMs). This is required to prevent the development of aGVHD that otherwise promotes resistance to tumor. The reduction of Treg cells establishes the GVT effect therapeutically after T cell-depleted (TCD) BMT. In conclusion, the GVT effect of CTC-BMT relies on a cross-talk between host tumor milieu and de novo Treg cells, required to expand TAMs compatible with M1 polarization. Significance: Tumor-driven host Treg cells promotes graft acceptance and expansion of donor TAMs that induce de novo CTLs after CTC-BMT, than donor-derived Treg cells after T cell-replete BMT, an immune reconstituted effect that confers either GVT effect or host tolerance to aGVHD, respectively.

P.C3.02.03
Preservation with portable ex vivo lung perfusion (EVL) reduces significantly ischemia/reperfusion injury in lung recipients by promoting cytotoxic antagonists
1MHH Institute of Transplant Immunology, Hannover, Germany, 2MHH Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover, Germany.
Objectives: The INSPIRE trial revealed significant reduction of PGD grade 3 using the Organ Care System (OCS) compared to the standard of care (SOC) for lung preservation. In order to investigate immunological mechanisms during these preservation processes, immune mediators were measured with the hypothesis that OCS preservation supports an anti-inflammatory milieu leading to reduced ischemia/reperfusion injury (IRI). Methods: Plasma, perfusates from 33 patients with OCS- vs. 26 with SOC preserved lungs were analysed for 95 immune mediators using multiplex assays. Recipient demographics, cold ischemic times (CIT), PGD scores at T0, T24 were assessed. Results: Clinical evaluation (OCS/SOC) revealed significant differences in recipient age and diagnoses. Cold ischemic time (549 vs. 258min) was significantly shorter in OCS group (p<0.0001). Improved PGD score was observed in OCS recipients (p<0.035). IL-6, CXCL8-10, plasma levels were reduced in OCS patients at T0 (p<0.01). IL-6 plasma levels of SOC recipients correlated with CIT, PaO2/FIO2 ratio and PGD >2 (all p<0.01). However, higher levels were observed in OCS vs. SOC perfusates (p<0.001). We proposed suppression of IRI by induction of antagonists. IL-1RA was significantly higher in OCS perfusates (p<0.05) and IL-31 correlated with IFN-g (p<0.001) only in OCS perfusates. Conclusion: Recipients of OCS-preserved lungs show significantly reduced IRI by reduced levels of pro-inflammatory immune mediators. Strong correlation of IL-6 with CIT and PGD in SOC but not OCS patients argues for its impact on IRI and early lung function. Thus, EVLP may have the potential to ameliorate IRI and improve clinical outcome by induction of cytokine antagonists.

P.C3.02.04
Silencing MHC expression on the organ’s endothelium decreases its immunogenicity and prevents a pro-inflammatory cytokine response
1Institute for Transfusion Medicine, Hannover Medical School, Hannover, Germany, 2Department of Cardiac-, Thoracic-, Transplantation- and Vascular Surgery, Hannover Medical School, Hannover, Germany.
Introduction: HLA mismatches are the main cause of graft rejection and failure. Methods: SLA class I and II expression was silenced on the lung endothelium using short hairpin RNAs-encoding lentiviral vectors. The effect of silencing MHC expression was evaluated in a porcine lung transplantation model by monitoring the cytokine response during 12 weeks. Immunosuppression was stopped 4 weeks after transplantation. SLA expression was silenced during normothermic ex vivo perfusion. Nanoluciferase was used as reporter gene. Levels of SLA were quantified by RT-PCR and flow cytometry. Cytokines were monitored every second day after Tx and weekly after the post-operative day (POD) 7 using multiplex technology. Results: SLA downregulation on lung endothelial cells reached a level of 70%. Already 1h after Tx the serum levels of IL-12/3bta, IL-6 and IL-8 increased significantly in all animals by up to 0.263, 1.370 and 0.497 pg/ml, respectively. On POD 1, the cytokine secretion in the recipient of SLA silenced grafts decreased to pre-transplant levels whereas those of the control group remained significantly elevated (p<0.01). On POD 14, levels of IL-12 increased significantly by up to 0.286 pg/ml in the control group whereas it remained at pre-transplant levels in the SLA silenced recipient lungs. In addition, levels of IL-2, IL-10 and TNF-α increased exclusively in animals with SLA expressing lungs while it was undetectable in animals receiving SLA silenced lungs. Conclusion: These data indicate that MHC-silenced grafts are less immunogenic and may combat the burden of rejection and immunosuppression.

P.C3.02.05
Liver resident mucosal-associated invariant T (MAIT) cells display an active but less proliferative state compared to the peripheral MAIT cells
W. Huang, W. He, X. Ye, Y. Gao;
The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China.
The liver is one of the most important immunological organs that remains tolerogenic in homeostasis. The composition of leukocytes in the liver is highly distinct from that of the blood and other lymphoid organs. In particular, the enrichment of innate T cells, i.e. invariant NKT cells (iNKT cells), Mucosal Associated Invariant T cells (MAIT cells). We performed flow cytometry on MAIT cells in blood and perfusion fluid from 20 patients undergone liver transplantation. Further functional assessments including MAIT cell proliferation, activation, and cytokine-producing capacity.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 371
A much higher percentage of MAIT cells were detected in the perfusion fluid compared with those in the PBMC (8.22% vs. 2.7%, p<0.001). In addition to that, the PBMC peripheral MAIT cells were in a less active state (lower CD69 expression) compared to their peripheral counterpart. Cytokines productivity post IL-12/IL-18 stimulation by MAIT cells were similar in both peripheral and perfusate as measured by the Luminex analysis. Interestingly, the live MAIT cells were found to be less proliferative upon stimulation with anti-CD3/CD28.

In here, we demonstrated that MAIT cells are numerically rich in the liver and functionally distinctive compared to their peripheral counterpart. These observations may be significant in transplant tolerance in the liver and other liver diseases.

P.C.3.02.06
Long-term graft tolerance induction by NPs: pathway inhibition in naive immune cells
L. Garnati1, C. Cigni2, F. Mingozzi2, L. Marongiu1, R. Rotem3, M. Colomba1, D. Prosperi1, I. Zanoni4, F. Granucci2
1Department of Biotechnology and Bioscience, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy, Milan, Italy, 2Harvard Medical School and Division of Gastroenterology, Boston Children’s Hospital, Boston, MA 02215, USA, Boston, United States.

Introduction: Transplantation is a therapeutic approach for failing organs, whose most feared complication is rejection, mediated by the instauration of alloreactive T cells, supported by the Nuclear Factor of Activated T cells (NFAT). To avoid graft loss, transplanted patients undergo lifelong administration of immunosuppressive drugs, completely abating the immune response. Since NFAT has recently emerged to be pivotal in sustaining innate immune cells, we aim at evaluating NFAT as a potential specific therapeutic target in these cells to prevent rejection without causing complete immune paralysis.

Methods: By taking advantage of nanoparticles (NPs) delivering a specific NFAT inhibitor peptide to innate immune myeloid cells, we managed to abolish NFAT activation in these cells without affecting T cells, in a model of mismatched skin graft.

Results: The administration of our NPs prevents acute allograft rejection. Accordingly, transplanting NFTaGKO skin, results in delayed rejection, due to the impaired vessels permeability and thus migration of T cells to the graft as well as of dendritic cells (DCs) to the draining lymph nodes. Moreover, we noticed a strong increase of CD4+ CD25+ Foxp3+ T cells in NPs-treated recipients, suggesting regulatory T cells-mediated tolerance against the graft. Accordingly, anti-NFAT NPs administration can be interrupted without provoking graft rejection. Diverse, if animals are treated with tacrolimus to inhibit rejection, therapy interruption immediately results in graft loss.

Conclusions: The inhibition of the NFAT signaling pathway in immune cells can be considered as a new approach to induce long-term graft acceptance without affecting T cell functions.

P.C.3.02.07
Induction and effector phase of human anti-pig T cell responses are differentially affected by the CRISPR/Cas9 induced absence of porcine MHC/SLA class-I molecules
R. Hein, S. Clever, H. Düvel, B. Trautewig, A. Brinkmann, J. Hundrieser, R. Schweizer;
Transplantation Laboratory, Clinic for General, Visceral and Transplantation Surgery, Hannover, Germany.

Introduction: Foreign MHC molecules display the main target for induction of anti-graft responses and rejection processes in the T-cell effector phase. By genetic engineering it is possible to generate immune cells and tissues lacking MHC expression which might particularly be suited for clinical xenotransplantation. Thus, we aimed to analyse how the absence of porcine MHC/SLA class-I (SLA, “swine leucocyte antigen”) affects the human anti-pig immune response. Therefore, a SLA class-I negative porcine cell line was generated and its stimulatory capacity to activate human T-cells was characterized in vitro.

Methods: SLA class-I deficient B cell line L23 (porcine APC, SLA-I, SLA-II) was generated using the Cas9 nuclease and a guide RNA directed against the beta2-microglobulin (b2m) coding gene.

Results: In flow cytometry analyses no b2m or SLA class-I expression was detected in L23-b2m-k0 cells; expression of SLA class-II and costimulatory molecules (CD40, CD80/86) was comparable to wildtype (wt) L23 cells. Proliferation of hPBMC triggered by L23-b2m-k0 cells was significantly reduced compared to L23-wt stimulation, mainly due to poor reactivity of hCD8+ T-cells. However, hCD8+ release of IFNγ showed protection of SLA class-I negative cells against cell-mediated lysis by human L23-specific cytotoxic T cells (generated in 6-day MLRs between hPBMC and L23-wt cells) and L23-b2m-k0 cells were lysed with similar intensity as L23-wt cells.

Conclusions: The induction phase of human anti-pig T cell reactivity can be diminished by the elimination of porcine SLA class-I molecules. However, alternative strategies will be required to protect porcine cells from human cytotoxic effector cells.

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P.C.3.02.08
In vivo and in vitro modulation of mTOR pathway facilitates human γδ T cells effector functions
H. Kaminski1, V. Pitard2, P. Merolle1, A. Terracine1, C. Larmorie1, I. Pellegrin1, R. Duran1, D. Dachau-Merville1;
1CNRS-UMR 5164 immunocompetence, Bordeaux, France, 2University hospital, Bordeaux, France, 3Inserm UMR 1218, Bordeaux University, Bordeaux, France.

In vivo and in vitro modulation of mTOR pathway facilitates human γδ T cells effector functions. mTOR pathway constitutes a crucial checkpoint in the activation of human γδ T cells. In vivo and in vitro modulation of mTOR pathway enhances γδ T cells effector functions. mTOR is constitutively activated in γδ T cells whereas its activity can be downregulated in vivo by rapamycin. mTOR inhibition promotes the cell proliferation and stimulates the expression of interleukin-2 and granzyme B. In vitro, mTOR inhibition promotes γδ T cell proliferation and γδ T cell cytotoxicity

P.C.3.02.09
Modulation of the IL-33-ST2 axis in regulatory T cell therapy
K. Kawai, M. Uchymama, J. Hester, F. Issa, K. J. Wood;
University of Oxford, Oxford, United Kingdom.

Background: Regulatory T cells (Tregs) are crucial mediators of immune homeostasis, with the ability to modulate alloreactive T cell responses and control transplant rejection. Previous work has shown that the modulation of the interleukin-33 (IL-33)/ST2 axis expands a highly suppressive subpopulation of Tregs. Here we present novel data that demonstrate the ability of exogenous IL-33 to induce Tregs in mouse models. IL-33 administration to mice with IL-33 knockout (IL-33 KO) resulted in a pronounced expansion of Tregs in the spleen. This expansion was associated with decreased CD8+ T cell infiltration into the graft, reduced graft rejection, and improved survival.

Materials/Methods: Recombinant IL-33 was administered to C57BL/6J mice with an established renal allograft. The mice were followed for 14 days, and splenocytes were collected for analysis. The expansion of Tregs was assessed by flow cytometry using CD4+ CD25+ Foxp3+ markers.

Results: Recombinant IL-33 administration induced Treg expansion in vivo, as evidenced by increased Foxp3+ Treg numbers in the spleen. Treg expansion was associated with decreased CD8+ T cell infiltration into the allograft, reduced allograft rejection, and prolonged graft survival.

Conclusions: The IL-33/ST2 axis is a potential target for modulating Treg expansion and improving graft survival, with potential implications for clinical transplantation.

P.C.3.02.10
BIUxx
K. Kotshwarova, M. Fialova, V. Svachova, L. Curnova, K. Vlasakova, O. Viklicky, I. Striz;
1,3IMMUNOLOGY department, Bordeaux university hospital, bordeaux, France,
2Renal transplant department, Bordeaux university hospital, Bordeaux, France,
3Inserm UMR 1218, Bordeaux University, Bordeaux, France.

Proinflammatory cytokines induced by ischemia/reperfusion injury upregulate release of chemokines from parenchymal cells to attract immune cells into transplanted kidney. The aim of our study was to evaluate the ability of human renal proximal epithelial cells (RPTEC) to produce multiple chemokines in response to TNF-α and compare the data with chemokine induction in renal adenocarcinoma (RA) and monocyte/macrophage (THP-1) cell lines. Concentrations of CXCL1, CXCL4, CXCL5, CXCL8, CXCL9, CXCL10, CXCL11, CXCL16, CCL2, CCL5, CCL18, CCL21 and CXCL1 were measured in culture supernatants by Luminex. Chemokines attracting neutrophils (CXCL1, CXCL5, CXCL8) were produced preferentially by TNF-α-stimulated epithelial cells. RT-PCR released more CXCL1 and CXCL8 while RA cells produced preferentially CXCL5. RT-PCR and RNA are also an important source of CXCL2, chemotactic for monocytes and other immune cells. In contrast, TNF-α-stimulated THP-1 cells produced more CCL5 (specific for monocytes, eosinophils and Th cells).
Chemokines attracting Tc and NK cells (CXCL13, CXCL14) and activated T cells (CXCL10, CXCL11) were preferentially induced by TNF-α in RTPE. We conclude that chemokines released from renal epithelial cells in response to TNF-α do not regulate only the influx neutrophils but are also involved in the recruitment of effector cell populations of adaptive immunity. Our data suggest, that primary epithelial cells are more convenient for studying chemokine regulation in kidney than renal adenocarcinoma epithelial cell line. Supported by Ministry of Heath of the Czech Republic, grant NR 15-26883A and by MH CZ-DRO (Institute for Clinical and Experimental Medicine - IKEM, IN 00023001).

PC.3.02.11
A novel way to generate human HLA class II monochlonal antibodies for HLA epitope analysis
C. S. M. Kramer1, M. E. Franke-van Dijk2, C. C. Zivold-van den Oever1, R. Rademaker1, P. W. Parren1, D. L. Roelen1, S. Heidt1
1Leiden University Medical Centre, Leiden, Netherlands, 2Gennemb, Utrecht, Netherlands.

Donor-specific antibodies produced upon renal transplantation are triggered by immunogenic epitopes present on mismatched donor HLA. However, not every foreign epitope will lead to antibody formation, which highlights the need to experimentally verify truly immunogenic epitopes. Human HLA-class II monochlonal antibodies (mAbs) have proven to be very useful for identification of HLA class II epitopes. Since currently the number of available HLA class II mAbs is limited, we aimed to produce these mAbs by using HLA class II-specific tetrameters and recombinant technology, of which here we show proof of principle. From PBMCs of an individual with HLA-DRB1*07:01-specific serum antibodies, single memory B cells positive for HLA-DRB1*07:01-specific tetramers were sorted (0.0012% of B cells). After expansion, 1/16 of B cell clones produced HLA-DRB1*07:01 antibodies, three also being reactive with HLA-DRB1*07:01. Subsequently, RNA was isolated to obtain the variable heavy and light chains to clone into pDnA3.3 expression vectors. Analysis of variable domain sequences identified B cells with different combinations of (V)DJ segments, suggesting that the isolated B cells originate from different precursors. At time of writing, we recombinantly expressed one HLA-DRB1*07:01-specific mAb and could this to confirm 25Q1, as an immunogenic epitope on HLA-DRB1. This study demonstrates that HLA tetramers can be used to isolate HLA class II-specific memory B cells and subsequently can be used as a source to generate recombinant mAbs. With this method a panel of various class II mAbs can be produced, to verify HLA epitopes and to perform studies on functional classes II antibodies.

PC.3.02.12
Redefining the HLA-DR11 peptide binding motif with new MS data
L. Labeur, A. C. Callado, Y. Arribas, R. Farroli, D. Jararquemada, M. Carrascal; Instituto de Biotecnología e Biomedicina-Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.

The HLA-DRB1*1101 (DR11) allele is associated to several diseases, being a protective allele for type 1 diabetes and systemic lupus erythematosus, while in multiple sclerosis it acts as a susceptibility agent. However, the last published data defining the DR11 motif is incomplete because of the low number of analysed peptides. Therefore, our objective is to accurately define this motif using a larger number of peptides eluted from DR11.

We cultured the human EBV-transformed HLA-DRB1*1101 homocytogen cell line BM21 and isolated Class II-epitope complexes by immunoprecipitation with the HLA-DR-specific B11.8 antibody. Peptides were eluted and then processed by MALDI and Orbitrap MS. Furthermore, we analysed the peptides previously eluted from thymus and spleen samples from DR11 donors. We thus observed that the sequences of peptides bound to human tissue match the motif obtained from cultured cells in vitro. In conclusion, contrary to previously described, although the main characteristics are maintained. All amino acids in P1 and P4 anchor positions were hydrophobic (F, L, I, Y and V, I, A, T, F respectively). In contrast, P6 was occupied by basic amino acids (R, K, and S), and P9 appeared to be irrelevant. Our data provide new information and add reliability to the current databases.

PC.3.02.13
IL-23/IL-17 pathway in kidney allograft rejection
Y. Lakhoua Gorgi1, Y. Haouami2, T. Dhouadi1, M. Jellouli1, J. Abdellatif1, M. Majdoubi1, M. Bacha1, R. Goucha1, R. Bardt1, Ben Abdellatif1, I. Sfar1
1Research Laboratory in Immunology of Renal Diseases (LRISRSP01), Tunis, Tunisia, 2Department of Medicine and Nephrology Charles Nicolle hospital, Tunis, Tunisia.

T helper cell 17 (Th17) subset has been implicated in autoimmune diseases, tumor immunity and transplant rejection. In order to investigate the role of interleukin 17 (IL-17F/17) in IL-23 pathway in allograft rejection, in vitro expression of IL-17 mRNA and protein (SNPs) of IL-17A, IL-17F, IL-17RC and IL-23R genes were evaluated with a quantification of plasma IL-17A, IL-17F, IL-23, and IL-23. This study revealed that recipients with acute rejection (AR) had a significant increase in IL-17A mRNA expression levels of after transplantation compared to controls (p<0.037). Moreover, plasma IL-17A levels were significantly higher in AR group; pre-transplantation (Day1): p=0.00022 and post-transplantation (Day7): p=0.014–14. IL-17F and IL-23 plasma levels were significantly higher in AR at Day7 only (7.86 vs. 22.99 pg/ml; and 33.82 vs. 18.811 pg/ml); p=0.015 and p=0.015–17, respectively. Using ROC curves, IL-17A and IL-23 plasma levels exhibited excellent sensitivities and specificities for predicting AR. Genetic study revealed no association between IL-17A, IL-17F, IL-17RC and IL-23R studied polymorphisms and AR. Nevertheless, plasma IL-17F levels at both Day 1 and Day 7 were significantly higher in patients carrying IL-17F-1507*C/T and IL-17F/T genotypes comparatively to those with the wild homozygous genotype *C/C, p=0.015 and p=0.022, respectively. Besides, IL-17A mRNA levels were significantly higher in patients carrying the IL-23R(G/G) genotype (3.68% 3.65) comparatively to those with *G/A genotype (180.367), p=0.042. Based on these findings, significant increase in IL-17A mRNA and protein levels in AR recipients which can be a useful clinical biomarker to predict early acute renal allograft rejection.

PC.3.02.14
Off-the-shelf™ mesenchymal Stromal cell therapy can prolong rejection-free survival of corneal allografts in a high immunologic risk transplant model by increasing regulatory cell populations
P. Lohan1, N. Murphy1, O. Treacy1, K. Lynch1, M. Marcois1, A. E. Ryan1, M. D. Griffin1, T. Ritter1
1Regenerative Medicine Institute, National University of Ireland, Galway, Galway, Ireland, 2Department of Pharmacology and Therapeutics, National University of Ireland, Galway, Galway, Ireland, 3CURAM Centre for Research in Medical Devices, School of Medicine, College of Medicine, Nursing and Health Sciences, National University of Ireland, Galway, Galway, Ireland.

Mesenchymal stromal cells (MSC) have been shown to be potent immunomodulatory and capable of prolonging corneal transplant (CT) survival. Patients with pre-existing allo-immunity or receiving a second CT are at higher risk of rejection and represent an unmet clinical need. A high-risk CT model was developed in rats by injecting the recipient with donor derived splenocytes 14 days before transplantation. Pre-sensitized CT recipients rejected significantly earlier (day 11.5) than animals which were not pre-sensitised (day 19.3) indicating they had pre-existing allo-immunity. Two pre-transplant intra-venous injections on days -7 and -1 of 1×1010 allogenic (to both donor and recipient) MSC were administered. This strategy significantly prolonged CT survival with 63.6% of MSC treated animals grafts surviving until day 30 compared to 0% in the untreated group. MSC treated animals had higher proportions (33.5%) of the immunomodulatory CD11b+ B220+ monocyctic cells in the lungs at the time of transplantation compared to untreated animals (7.6%). At the average time of rejection in untreated animals (day 10), MSC treated animals also had a higher proportion of CD4+ CD25+ FoxP3+ Tregs (7.9%) in their draining lymph node compared to untreated animals (4.4%). Finally, CD11b+ c/ cells isolated from the lungs of naïve rats were shown to generate more Treg in vitro after being co-cultured with MSC than those not exposed to MSC. This work sheds light on a potential mechanism of action and underlines the utility of MSC in immunological diseases and particularly in high risk CT.

PC.3.02.15
In vitro TGF-β secretion capacity of stimulated peripheral blood cells correlates with the occurrence of opportunistic infection in liver and kidney transplantation
F. Boix, G. Gonzalez-Martinez, R. Affian, I. Legaz, R. Moya-Quiles, A. Minguela, J. Pons, J. Gallan, S. Llorente, M. Muro; Immunology Service, Clinical University 'Vergel Aragonia'- IMIB, 3510 Murcia, Spain, Murcia, Spain.

Introduction: TGF-β has been known to act as a potent immune-regulatory cytokine, which blocks T-cell activation. It is considered as a potential target for more specific and less toxic immunosuppression and control of alloimmune-responses over the long term in transplantation in this setting, TGF-β main function after SOT remains yet unclear and is important to further characterise its role under different clinical circumstances not only for operational tolerance, but also amongst other comorbidities such as opportunistic-infections (OI).

Objectives: The aim of this study was to assess the concentration of TGF-β in the supernatant of stimulated WPB in a cohort of SOT recipients (SOTr) and correlate it with the primary study outcome which was the occurrence of OI. Material and Methods: 30 liver(LT) and 31 kidney(KT) transplant recipients as well as 15 healthy volunteers (HCs) were recruited and prospectively monitored for one year in our Hospital. ELISA was carried out to calculate TGF-β concentration after WPB culture with Concanavalin A for 72 hours. Results: In this cohort, SOTr showed higher TGF-β concentration compared to HCs. The stratification analysis showed that TGF-β was significantly higher in patients with OI within the first six months following transplantation. A TGF-β >363.25 ng/ml in LT and >808.51 ng/ml in KT were transplanted to be the most accurate cut-off values to stratify SOTr at high risk of OI. The regression model confirmed this biomarker as the main recipient risk factor for developing OI. Discussion: Our data show that the quantification of TGF-β could provide valuable information as to the occurrence of OI in LT and KT.
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P3.C3.02.16

Poster Presentation of Alloreactive T Cells in Peyer’s Patches: Tracking the Homing of Highly Proliferative Cells in vivo

K. J. Ottmüller1, Z. Makokri2, L. Scheller1, J. Hartweg3, S. Thwee1, D. Le4, M. Ronesczy5, H. Shaikh2, M. Mareschi6, K. G. Heinze5, A. Beilhack1,2,4,7

1University Hospital Würzburg, Würzburg, Germany;
2Graduate School of Life Sciences, University of Würzburg, Würzburg, Germany;
3Rudolf Virchow Center, University of Würzburg, Würzburg, Germany;
4Department of Pediatrics, University Hospital Würzburg, Germany.

Tracking and marking of T cells is important to study their trafficking, which is an elementary process to all immune reactions. Only with this efficient homing, single immune cell clones can convey protection against intruders or regulate immune responses throughout the whole body. Photoconversion is a superior labeling technique for intravital application because it enables contactless time- and site-specific marking of cells in the tissue without surgically manipulating the microenvironment. However, the converted fluorescent protein may decline quickly in proliferating cells.

We demonstrate the suitability of photoconversion to tracking highly proliferating T cells from the priming site of T cell activation to peripheral target organs. Dendra2 T cells were photoconverted in the Peyer’s patches during the initiation phase of acute graft-versus-host disease (GVHD). We tracked these cells through the mesenteric lymph nodes and the primary effector site, the small intestine, with flow cytometry and intravital two-photon microscopy.

Photoconverted alloreactive T cells preserved the full proliferative capacity and cytotoxicity against tumor cells. We quantified the trafficking cells throughout their homing route and observed that their migration in the intestinal lamina propria was retained after photoconversion. We conclusively proved that photoconversion of highly proliferative alloreactive T cells in the Peyer’s patches is an effective tool to study trafficking of alloreactive T cells under physiological conditions and to GVHD target tissues. This technique can also be applied to the study of immune cell tracking under inflammatory and non-inflammatory conditions.

P3.C3.02.17

Dendritic cells are affected by VEGF depletion leading to corneal graft acceptance

A. Schneider1, E. Zinszer1, F. Bock;

1Department of Ophthalmology Cologne, Cologne, Germany;
2Department of Immunomedulation, Erlangen, Germany.

The main risk factor in corneal transplantations is the presence of blood and especially lymphatic vessels in the normally avascular and immune-privileged cornea. In the murine model of high risk corneal transplantation neovascularization can be induced by a sterile inflammation caused by intrastromal corneal sutures. VEGF-depletion during this inflammation prevents vessel ingrowth into the cornea, which significantly improves subsequent graft survival.

Dendritic cells (DCs) are the main immune cells that are trafficked through lymphatic vessels and are responsible for antigen uptake and subsequent activation of T cell mediated rejection in transplant settings. We demonstrate that VEGF-depletion does not only act on vessel formation, but has an immune modulatory effect on DCs. Treatment of DCs with VEGFR1/2 Trap affected costimulatory molecule expression, cytokine secretion and impaired the capacity of the cells to stimulate T cells in vitro. VEGF depletion in mice during suture induced neovascularization impairs recruitment of DCs into the cornea and weakens trafficking of DCs. In addition we could show that VEGF-depletion acts in an immune modulatory manner on the DCs directly through decreased costimulatory molecule expression and subsequent induction of more potent CD4+CD25+Foxp3+ regulatory T cells.

P3.C3.02.18

CD40 ligation on monocyte derived dendritic cells enhances the production of the positive complement regulator propeptide

M. F. van Essen, N. Schlegewin, J. M. Ruben, C. van Kooten, on behalf of the COMBAT Consortium;

Div. of Nephrology and Transplant Medicine, Dept. of Medicine, Leiden University Medical Center, Leiden, Netherlands.

The important role of local complement production and activation has been demonstrated in experimental transplantation. During APC-T cell interactions, C3a and C5a contribute to T cell proliferation and cytokine production by APCs. Propeptide (PP) stabilizes the C3 convertase, thereby enhancing C3a and C5a generation. We investigated the regulation and functional consequence of IP production by monocyte derived dendritic cells (MoDCs) upon IP stimulation or the mimic of co-stimulatory signals in T cell-APC interaction CD40L. MoDCs were stimulated for 48h with either LPS, CD40L expressing L-cells or control cells. CD40L induced a dose-dependent increase in IP production (mean 7.5-fold increase, 18 ng/ml, n=10), compared to controls (mean 2.5ng/ml). LPS stimulation increased IP levels as well (mean 15ng/ml, n=10). The combined activation with CD40L and LPS did not result in synergistic IP production, whereas this was observed for IL-10 and IL-12p70.

To explore the functional role of IP, MoDCs were treated with a siRNA-pool against IP at day 5 and additionally cultured for 48 hours with or without stimulation. At day 7, cells were harvested and co-cultured with CFSE-labelled allogeneic T cells. PP knockdown was confirmed by ELISA and exposure of immature MoDCs to allogeneic T cells led to a reduced T cell proliferation, as determined by FACS.

In conclusion, LPS and CD40 activation increases IP production by MoDCs. Since CD40L is specifically expressed on activated T cells, this introduces cognate specificity to this local production of IP.

P3.C3.02.19

Mechanisms of calcification in human bio-devices

A. Pauf1, S. Ben-Arye2, L. Govani1, H. Yif1, F. Fellah-Hebia1, M. Pascual-Gilabert1, C. Costa1, R. Mallec1, M. Gililachines1, B. Tamasa2, G. Cesana1, J. Roussel1, T. Le Tourneaux1, J. Soullieu1, X. Chen1, L. Liq1, G. Gerosa2, E. Cozzi3, V. Padera-Karvanovi1;

1Tel Aviv University, Tel Aviv, Israel;
2Department of Chemistry, University of California-Davis, Davis, CA, United States;
3Institut du Thorax, Department of Thoracic and Cardiovascular Surgery, University of Bordeaux, INSERM UMR1087, Nantes, France;
4Infectious Diseases and Transplantation Division, Bellvitge Biomedical Research Institute (IDIBELL), L’Hospitalet de Llobregat, Barcelona, Spain;
5Department of Cardiovascular Research, The Heart Institute, Hospital Universitari Vall d’Hebron and Vall d’Hebron Research Institute, Barcelona, Spain;
6Cardiovascular Regenerative Medicine Group, Department of Cardiac, Thoracic and Vascular Surgery, University of Padua, Padua, Italy;
7Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (ISCIII), Madrid, Spain;
8Department of Chemistry, University of California-Davis, Davis, CA, United States;
9Venetian Institute of Molecular Medicine, Regenerative Medicine Group, Department of Cardiac, Thoracic and Vascular Surgery, University of Padua, Padua, Italy.

The important role of local complement production and activation has been demonstrated in experimental transplantation. During APC-T cells interactions, C3a and C5a contribute to T cell proliferation and cytokine production by APCs. Propeptide (PP) stabilizes the C3 convertase, thereby enhancing C3a and C5a generation. We investigated the regulation and functional consequence of IP production by monocyte derived dendritic cells (MoDCs) upon IP stimulation or the mimic of co-stimulatory signals in T cell-APC interaction CD40L. MoDCs were stimulated for 48h with either LPS, CD40L expressing L-cells or control cells. CD40L induced a dose-dependent increase in IP production (mean 7.5-fold increase, 18 ng/ml, n=10), compared to controls (mean 2.5ng/ml). LPS stimulation increased IP levels as well (mean 15ng/ml, n=10). The combined activation with CD40L and LPS did not result in synergistic IP production, whereas this was observed for IL-10 and IL-12p70.

To explore the functional role of IP, MoDCs were treated with a siRNA-pool against IP at day 5 and additionally cultured for 48 hours with or without stimulation. At day 7, cells were harvested and co-cultured with CFSE-labelled allogeneic T cells. PP knockdown was confirmed by ELISA and exposure of immature MoDCs to allogeneic T cells led to a reduced T cell proliferation, as determined by FACS.

In conclusion, LPS and CD40 activation increases IP production by MoDCs. Since CD40L is specifically expressed on activated T cells, this introduces cognate specificity to this local regulation. This places IP in addition to C3a and C5a, as a new regulator promoting APC-T cell activation.

P3.C3.03.01

Organ Transplantation, Genotyping

P3.C3.03.01

High intensity de novo donor specific HLA class II antibodies associated to renal chronic rejection

R. Alendo1, M. Moreno-Hidalgo1, A. Balas1, F. Garcia-Sanchez1, L. Barea1, M. Rodriguez-Ferrer2, F. Ana2, J. Vicario1;

1Centro de transference de la comunidad de Madrid, Madrid, Spain;
2Hospital Universitario Gregorio Marañon, Madrid, Spain.

Introduction: Development of de novo donor-specific anti-HLA antibodies (dnDSA) is associated with reduction in renal graft survival. High titer (>5000 MFI) of anti-HLA class II antibodies is frequently found in chronic rejection. The aim of the study was to analyze the incidence of dnDSA, their targets/titer, and clinical relevance. Material and Methods: The study included 393 renal transplants from 2010 to 2018 at a single center. Seras were tested using Lifecodes Class I and Class II SAB kits (Immunor, USA). Results: 44 patients (11.4%) developed dnDSA in 144 months as average. 5 patients developed antibodies only against class II (114 months). 11 patients against class II (3.6%) and 28 patients against both (9.2%). The mean of the high titer reached for anti-HLA class I antibodies was 4833 MFI, anti-HLA class II DRB11123 MFI, anti-HLA class II DQB1 10258 MFI and anti-HLA class II DQA1 9890 MFI. Conclusion: 14.5% of the patients studied develop dnDSA in the follow-up. The percentage of patients with anti-HLA antibodies against class II antigens is higher, and they react with higher maximum titer than anti-HLA class I antibodies. The highest antibody response seems to be directed against immunodominant epitopes.
POSTER PRESENTATIONS

P.C3.03.02

Relationship between adaptive NK cell markers and the incidence of cytomegalovirus infection in kidney transplant recipients

M. Ataya1, D. Redondo-Pachón2, L. Líñez3, J. Vilamos1, G. Heredia1, L. Sorai4, J. Pascual3, M. Crespo1, M. López-Boer1,4

1Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain; 2Nephrology S. Hospital del Mar, Barcelona, Spain; 3Immunology S. Hospital del Mar, Barcelona, Spain; 4Univ. Pompeu Fabra (UPF), Barcelona, Spain.

Human cytomegalovirus (HCMV) infection has been related with an increased risk for graft loss and reduced host survival in kidney transplant recipients (KTR). HCMV infection may promote the adaptive differentiation and persistent expansion of an NKG2C+ NK cell subset. These adaptive NK cells are functionally mature, efficiently mediate specific antibody-dependent cellular cytotoxicity (ADCC), and display a variable extent discrete phenotypic features, reflecting late differentiation events (e.g. CD57 and ITT2 expression; FcRγ chain downregulation). In a prospective study of a KTR cohort (N=122), high pre-transplant levels of NKG2C+ NK cells were found associated with a reduced incidence of post-transplant HCMV viremia. We have extended the phenotypic analysis by multiparametric flow cytometry in available cryopreserved pre-transplant PBMC samples from cases of the same KTR cohort. The expression of NK2DA2, CD57, ILT2 (IRB1) and FcRγ-Y was analysed in NK cells and T lymphocytes, including TCRγδ+ subsets [Vδ2+ and Vδ2–]. Individually, these markers appeared unrelated with post-transplant HCMV infection. Yet, when their co-expression with NKG2C+ in NK cells was considered, significantly lower proportions of NKG2C– NK2DA2+, CD57+, ILT2+, ILT2–, FcRγY+ and FcRγY– were detected in KTR developing HCMV infection (Wilcoxon test). These results, together with CMV-free survival analysis (Kaplan-Meier), further support the relationship between NKG2C+ NK cells and the risk of post-transplant HCMV infection.


Ex vivo lung perfusion affects the immunological milieu and regulatory T cells in a porcine lung transplantation model

R. Bellmás Sàn1, J. Solana1, T. Siemens2, K. Jansson1, A. Knöfel1, F. Ius1, K. Höffner1, A. Haverich1, I. Tudorache1, C. S. Falk1, G. Warnecke1, N. H. Litjens2, B. Wiegmann2, K. Jansson1, A. Knöfel1, C. Neudörfl1

1Institute of Transplant Immunology, Hannover Medical School, Hannover, Germany; 2Department of Cardiothoracic, Vascular and Transplantation Surgery, Hannover Medical School, Hannover, Germany.

Introduction: Ex vivo lung perfusion (EVLU) is an alternative to cold static storage for graft preservation in lung transplantation. Here, we investigate mechanisms of improved preservation using the Organ Care System (OCS) as EVLU platform, focusing on immunological changes in a porcine lung transplant model. Methods: 12 porcine lungs were explanted from healthy donors: five lungs were preserved for 6h in the OCS (OCS group) and six lungs on ice (standard of care, SOC group). The left lungs were transplanted into allogeneic porcine recipients and all pigs were observed for 6 hours after clamping the contralateral lung. We investigated the presence of cytokines and Treg, Tnon subunits in recipient blood, bronchoalveolar lavage (BAL) fluid and perfusates. Results: In BAL of OCS recipients at 6h, significantly lower levels of IL-6 (p=0.04), IL-1α (p=0.04), IL-19 (p=0.03), IL-12 (p=0.03), IL-18 (p=0.03) and IFN-g (p=0.04) were observed compared to SOC recipients, which was accompanied to increased expression of CD25 on CD4+ and CD8+ T cells (p=0.03). In perfusates of OCS lungs, significantly higher pro-inflammatory cytokine levels were detected compared to perfusates of SOC lungs, especially the antagonist IL-19. Remarkably, in these OCS perfusates, FoXP3 expression of CD4+CD25+ T cells was maintained but significantly decreased in SOC perfusates (p=0.001). Conclusions: Preservation using the OCS has a strong immunological impact towards an anti-inflammatory milieu systemically and maintenance of FoXP3 expression in Treg reflecting human data of the INSPIRE trial.

P.C3.03.04

A unique CD56dimNK cell subset, which is also present in lung perfusates, increases in the periphery of lung transplant recipients

R. Bellmás Sàn1, M. Seyda1, B. Wiegmann1, K. Blassing1, C. Neudörfl1, A. Knöfel1, I. Tudorache1, K. Höffner1, M. Ausor1, A. Haverich1, G. Warnecke1, C. S. Falk1

1Institute of Transplant Immunology, Hannover Medical School, Hannover, Germany; 2Department of Cardiothoracic, Vascular and Transplantation Surgery, Hannover Medical School, Hannover, Germany.

Purpose: Biomarkers to predict short and long term outcome after lung transplantation are urgently needed. Monitoring of the longitudinal dynamics lymphocyte subsets after lung transplantation represents a feasible strategy for the identification of potential biomarkers for rejection. In this study we defined the phenotype and kinetics of NK and T cell subsets in recipient blood and compared them with their counterparts in perfusates. Methods: Perfusion solutions and blood were obtained before (pre Tx), directly after (T0), 24h (T1) and 3 weeks post transplantation from 60 lung transplant recipients. T and NK lymphocyte subsets from peripheral blood and perfusates were analysed by multicolor flow cytometry. Results: At T0, NK cells increased in recipient blood compared to pre Tx values (p=0.01) and declined at T24 (p=0.04), whereas T cells decreased at T0 (p=0.005) and recovered at T24. The phenotype of these NK cells consisted of CD56dimCD16+161 cell subsets, with high KIR NK cell proportions and significantly elevated CD69 and CD25 expression. T cells at T0 were also enriched for CD69+, CD25+ and KIR1,2 subsets. This was the prevalent T and NK cell phenotype in perfusates, a unique compartment containing T and NK cell subsets significantly different from matched PBMCs. Conclusion: After lung transplantation, CD56dim NK cells with a unique phenotype increase in recipient blood, which raises the question whether NK cells are recruited rapidly into the periphery and/or donor cells are migrating out of the transplanted lung. The similarity of these cells with perfusate cells may indicate their donor origin.

P.C3.03.05

Multiplex KIR and HLA class i genotyping using Next Generation Sequencing

L. Closa1, F. Vidal1,2, M. J. Herrero1, J. L. Caro1

1Institute of Transplant Immunology, Blood and Tissue Bank, Barcelona, Spain; 2Transfusion Medicine Group, Vall d’Hebron Research Institute, Autonomous University of Barcelona (VHIR-UAB), Barcelona, Spain; 3Congenital Coagulopathy Laboratory, Blood and Tissue Bank, Barcelona, Spain; 4CIBER of Cardiovascular Diseases (CIBERCV), Madrid, Spain.

The killer cell immunoglobulin-like receptors (KIR) are considered the most polymorphic Natural Killer (NK) cell regulators, binding HLA class-I molecules or still unknown ligands. Lately, the interest on KIR genotyping has increased as it has been shown their importance in the identification of the best possible donors for Hematopoietic Stem Cell Transplantation (HSCT) to obtain graft-versus-leukemia effect. Currently, protocols to determine gene content of KIR cluster are exclusively performed by PCR-SSO and PCR-SSP. To improve the study of these genes, we developed a multiplex Long-Range PCR strategy suitable for simultaneous high-resolution HLA class I and KIR genotyping by Next Generation Sequencing (NGS). This protocol allows the amplification of 17 KIR genes and pseudogenes and HLA class I with further sequencing using an Illumina sequencer. The bioinformatics analysis for KIR genotyping was performed using in-house gene-specific virtual probes by CLC Genomics Workbench 11 and the HLA genotyping by GenDx NGGenome software 2.8.0. To validate the method reliability, 96 previously characterized genomic DNA samples were used. When specific KIR was present, an average of 415 gene-specific virtual probes was detected, meanwhile when it was absent, the average was 6, facilitating the cutoff establishment. Also, the rate of concordance for both KIR and HLA was 100% compared with previous results. In conclusion, we demonstrated that the multiplex PCR NGS-based strategy could provide a much more efficient and economic method for KIR ligand genotyping at presence-absence level compared to current techniques. Moreover, reach the allelic level will be possible when specific software becomes available.

P.C3.03.06

Immunosuppressive drug withdrawal late after liver transplantation leads to an improvement of lipid metabolism, a reduction of infections and an increase in gamma-delta Vδ1 perforin and granzyme B positive T-cells

A. A. Duizendstra1, R. J. de Knecht2, M. G. Betjes3, N. H. Litjens3, J. Kwekkeboom3

1Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, Netherlands; 2Department of Internal Medicine - Division of Nephrology and Transplantation, Erasmus University Medical Center, Rotterdam, Netherlands.

Background: Liver transplant (LTx) recipients need lifelong treatment with immunosuppressive drugs (IS) to prevent rejection. Long-term use of IS is accompanied by adverse effects. Occasionally LTx recipients can be fully withdrawn from IS and are considered to be tolerant towards their graft. Studies indicate that the V61/S52 ratio of γδT-cells is a useful biomarker to identify tolerant LTx recipients. Objectives: To assess clinical and immunological effects of IS withdrawal late after LTx. Methods: LTx recipients who were withdrawn from IS (IS withdrawal group on IS [ISWL]; n=22) matched for time after LTx, age, gender and CMV serostatus were included. Liver and kidney function, lipid profile and infections before and after withdrawal or matching time points were evaluated. PBMCs were characterized by flow-cytometry. Results: Liver function (bilirubin, AST, ALT) levels significantly improved in TOL after IS withdrawal, whereas kidney function did not. LDL levels and total number of infections significantly decreased in TOL after IS withdrawal, but not in CTRL. No differences were found in V61/S52 ratios between both groups, but V61+GranzymeB+Perforin+ γδT-cells were significantly increased in TOL compared to CTRL. Conclusion: After IS withdrawal no deterioration of graft function is observed, lipid metabolism improves and total number of infections decreases, but kidney function does not improve. It is likely that kidney damage in LTx recipients is irreversible after long-term IS therapy. V61/S52 ratio is not increased in TOL, which may be due to matched CMV serostatus of CTL, but in TOL enhanced cytotoxic status of V61 γδT-cells is observed.
Alloantibody can detect a single amino acid change at position 59 in HLA-B27

N. Egri Cidrada, M. Digon Doral, A. Manchon Castillo, E. Palou Rivera, J. Martorell Pons;
Department of Immunology, Hospital Clinic of Barcelona, Spain.

Abstract: Background: HLA antibodies identification by SAB evidences diversity of alloantibody reactivity between alleles with a common first field but different second field (e.g. B*27:03, B*27:05, B*27:08) corresponding to different proteins of the same "serological specificity".

Objectives: To quantify and analyze the differences in reactivity between different HLA-B27 proteins with common first field but different second field.

Methods: Sera from 1,100 patients on kidney transplant waiting list were analyzed with KIT Lifecodes LSA Immuno. 304 had anti-HLA-1. Those positive with at least one B*27 protein were selected. Patterns had been quantified and their rationale was analyzed at the epitope and sequence level.

Results: We found 66 patients with some reactivity with B*27 alleles. Surprisingly, 14/66 (22%) had the pattern (B*27:03, B*27:05, B*27:08) not explained by described eplets. Allele B*27:03 has a discernible discrepancy with B*27:05 and B*27:08 in position 59 Histidine/Tyrosine that should be explained by this pattern. The rest of the patterns are explained by several eplets as (B*27:03, B*27:05, B*27:08) explained by verified 65QDA, 69AQA, 80TLR, 82LR, 1315, 163EW or non verified 66IC, 71KA, 76ID, 102DV, 156LA, 170RY, 193PI (37/66); (B*27:03, B*27:05, B*27:08) explained by 765Q (7/66); (B*27:03, B*27:05, B*27:08) explained by 80TLR, 82LR (7/66). Probably pSHM can modify reactivity with eplets 65QDA, 69AQA and 163EW that are in a short distance in tridimensional structure.

Conclusions: A single change at position 59 Histidine/Tyrosine can explain differences in alloantibody reactivity in 22% of B*27 reacting patients. This change is not involved in described eplets.

Corneal infiltrating lymphocytes in corneal resection: pilot study

F. Esen1, E. Cetin1, S. Genci1, G. Deniz1, M. Taskapili1, M. Oguz2;
1Istanbul Medeniyet University School of Medicine, Department of Pathology, Istanbul, Turkey; 2Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey.

Corneal transplantation is the most common transplantation procedure globally and corneal resection is still an important complication limiting its success. The aim of this study was to analyze infiltrating lymphocytes in patients with corneal resection.

Two patients who needed graft exchange due to corneal resection were included into the study. Corneal infiltrating lymphocytes were isolated from tumor dissociation kit with gentle MACS Dissociator and were analyzed by flow cytometry. The sample of the first patient was stained with anti-human CD45-FITC/CD14PE, anti-human CD3-FITC/CD19-PE and the second sample was stained with CD45-FITC/CD14-PE, anti-human CD3-FITC/CD10-PE and monoclonal antibodies.

The first case was a 36-years-old, male, keratoconus patient who had keratoplasty 8 years ago and the second case was a 23-years-old, male, keratoconus patient who had keratoplasty 3 years ago. Both patients needed rekeratoplasty due to corneal resection and opacification, which was unresponsive to medical treatment. The flow cytometry analysis revealed that 33% of the infiltrating cells were CD3+T and 17% were CD19+B lymphocytes. Among the CD3+ T lymphocytes, expression of CD4 was 19.7%.

This pilot study demonstrated that corneal infiltrating lymphocytes could be isolated from human corneal tissue with kits designed to extract lymphocytes from other solid tissues. Previous animal model work and human aequous humor studies showed that T cell and B cell cooperation in corneal rejection in rodent transplantation model. Further work on this topic would contribute better understanding of the immunology of human corneal resection.

Corneal infiltrating lymphocytes in corneal resection: pilot study

K. Genevegelli1, M. Niemann1, J. Drylewicz1, H. G. Otten1, E. Spierings1, on behalf of the PROCARE consortium;
1Laboratory of Translational Immunology, UMC Utrecht, Utrecht, Netherlands; 2PIRCHÉ AG, Berlin, Germany.

Individual HLA mismatches may differentially impact graft survival after kidney transplantation. Therefore, there is a need for a reliable tool to define permissible HLA mismatches in a kidney transplantation setting. We previously demonstrated that donor-derived HLAs with a micro-mismatch in the fourth Igv domain presented by recipient HLA class II (PIRCHÉ-II) play a role in de novo DSA formation after kidney transplantation. In the present Dutch multi-center study we evaluated the possible association between PIRCHÉ-II and kidney graft failure in 2,918 donor-recipient pairs that were transplanted between 1995 and 2005. For these donors-recipients couples, PIRCHÉ-II numbers were related to graft survival in univariate and multivariate analyses. Adjusted for confounders, the natural logarithm of PIRCHÉ-II was associated with a higher risk for graft failure (HR:1.13, 95% CI:1.04-1.23, p=0.003).

When analyzing a subgroup of patients who had their first transplantation, the hazard ratio of graft failure for in(PIRCHÉ-II) was higher compared to the overall cohort (HR:1.22, 95% CI:1.10-1.34, p<0.001). PIRCHÉ-II demonstrated both early and late effects on graft failure in this subgroup. These data suggest that the PIRCHÉ-II may impact graft survival after kidney transplantation. Inclusion of PIRCHÉ-II in donor-selection criteria may eventually lead to an improved kidney graft survival.

PIRCHÉ-II is related to graft failure after kidney transplantation

P. C3.03.07

Corneal infiltrating lymphocytes in corneal resection: pilot study

P. C3.03.08

Corneal infiltrating lymphocytes in corneal resection: pilot study

P. C3.03.09

Corneal infiltrating lymphocytes in corneal resection: pilot study

P. C3.03.10

Corneal infiltrating lymphocytes in corneal resection: pilot study

P. C3.03.11

Corneal infiltrating lymphocytes in corneal resection: pilot study

P. C3.03.12
P.C3.03.14 Detection of a new HLA-A*30 allele in a donor from the NMGR register

M. Villches-Moreno, M. San Jose-Cascón, E. Garcia-Moreno, A. Nieto; UGC Hematology, Immunology and Genetics, Hospital Universitaria Puerta del Mar, Cádiz, Spain.

Introduction: High resolution typing for HLA molecules is a crucial component in donor unrelated stem-cell transplantation. Mismatches between patient and donor may lead to transplantation-related complications resulting in morbidity or mortality. Here we describe the identification of a novel HLA-A*30 null allele in the course of high resolution confirmatory typing that precluded donation from an a priori matched unrelated bone marrow donor.

Methods and Materials: HLA alleles were sequenced through exons 2-4 in both directions using reagent kit AlleleSEQR HLA and analysed with Assign SBT software. Primers were designed to separately amplify and sequence the third exon of HLA-A*30 and HLA-A*01. A PCR-SSP strategy was designed to further confirm the presence of the new allele.

Results: Typing of the sample by registry was A*01:11;12, A*30:PPPW. The sequence obtained using HLA-A AlleleSeqr kit did not fully match with any allele pairs in the IMGT/HLA database and showed two heterozygous mismatches with respect to HLA-A*01:01;30:01 allele pairs. Specifically, codon 175 showed G/T at position 3 and codon 176 showed T/A at position 3 (in bold type). The presence of these changes was ascertained both by sequencing exon 3 of A*01 and A*30 separately from each other and by in house designed PCR-SSP. Both approaches showed that the mismatched TT were in the A*30 allele. The change in codon 176 generates a stop codon (TAG) in the A*30 allele most probably abolishing its surface expression.

Conclusions: We have found a probably new HLA-A*30 allele with sequence variations not described so far.

P.C3.03.15 Prevalence and impact of preformed and de novo anti-HLA donor specific antibodies in liver transplantation

M. Papachristou, A. Fykstas, M. Daoudaki, E. Chalongtis, T. Karampatos, A. Anastasiou, A. Sarantopoulos, G. Chatzikir, L. Vagiotas, I. Fouzas; 1National Peripheral Histocompatibility Center-Immunology Department, Hippokration General Hospital, Thessaloniki, Greece, 2Biochemistry Laboratory, Aristotle University of Thessaloniki, Medical School, Thessaloniki, Greece.

Introduction: High resolution typing for HLA molecules is a crucial component in donor unrelated stem-cell transplantation. Mismatches between patient and donor may lead to transplantation-related complications resulting in morbidity or mortality. Here we describe the identification of a novel HLA-A*30 null allele in the course of high resolution confirmatory typing that precluded donation from an a priori matched unrelated bone marrow donor.

Methods and Materials: HLA alleles were sequenced through exons 2-4 in both directions using reagent kit AlleleSEQR HLA and analysed with Assign SBT software. Primers were designed to separately amplify and sequence the third exon of HLA-A*30 and HLA-A*01. A PCR-SSP strategy was designed to further confirm the presence of the new allele.

Results: Typing of the sample by registry was A*01:11;12, A*30:PPPW. The sequence obtained using HLA-A AlleleSeqr kit did not fully match with any allele pairs in the IMGT/HLA database and showed two heterozygous mismatches with respect to HLA-A*01:01;30:01 allele pairs. Specifically, codon 175 showed G/T at position 3 and codon 176 showed T/A at position 3 (in bold type). The presence of these changes was ascertained both by sequencing exon 3 of A*01 and A*30 separately from each other and by in house designed PCR-SSP. Both approaches showed that the mismatched TT were in the A*30 allele. The change in codon 176 generates a stop codon (TAG) in the A*30 allele most probably abolishing its surface expression.

Conclusions: We have found a probably new HLA-A*30 allele with sequence variations not described so far.

P.C3.03.16 Analysis of monocyte derived macrophages from lung transplantation patients

I. Schreurs, B. Meek, C. van Mooresele, H. D. Luikj, J. M. Kwakkel-van Erp, E. Oudijk, D. van Kessel, J. C. Grutters; 1Sint Antonius Hospital, Nieuwegein, Netherlands, 2UMC Utrecht, Utrecht, Netherlands.

Lung transplantation (LTx) is a last treatment option for patients with an end-stage pulmonary disease. Standard immunosuppressive medication is mainly focused on the acquired immune system. How this medication affects the monocyte-macrophage lineage is not known. The goal of this study is to determine how monocyte subsets and differentiation towards macrophages are affected in LTx patients. Monocytes were isolated from whole blood samples that were analysed and monocyte subsets identified using flow cytometry. To obtain macrophages, monocytes were differentiated in vitro. We aimed to see whether pre-derived macrophages, PBMCs were collected from LTx patients and matched controls. Monocytes were differentiated and macrophage phenotype and cytokine production were analysed. Total peripheral monocyte numbers were decreased in LTx patients compared to healthy controls. The ratio between monocyte subsets showed a shift with increased classical monocytes and decreased non-classical monocytes. Surface marker expression levels of TLR2, CD163 and CD36 were increased and CD86 was decreased. Experiments analysing macrophage phenotype and cytokine production in macrophages are currently in progress. The differences found in monocyte count and expression of surface markers are thought to impact a shift towards an M2 macrophage in LTx patients. In vitro confirmation is ongoing.

P.C3.03.17 The function of external respirationin patients after kidney transplantationin condition of immunosuppressive therapy

M. Papachristou, A. Fykstas, M. Daoudaki, E. Chalongtis, T. Karampatos, A. Anastasiou, A. Sarantopoulos, G. Chatzikir, L. Vagiotas, I. Fouzas; 1National Peripheral Histocompatibility Center-Immunology Department, Hippokration General Hospital, Thessaloniki, Greece, 2Biochemistry Laboratory, Aristotle University of Thessaloniki, Medical School, Thessaloniki, Greece.

Introduction: One essential problem in the waiting list for kidney transplantationare hyperimmunized patients. The high sensitivity of solid-phase techniques for the study of antibodies and definition of viral crossmatch make transplant difficult in them. Therefore, it should be considered the possibility of receiving grafts looking for assumable risks depending of fluorescence intensity values of DSAs.

Methods and Material: We followed 41 patients with a mean of 47.48 months in waiting list and average panel reactive antibody (PRA) of 52.4% transplanted 2014-2017 with maximum CDC-XM and positive virtual-VM (maximum allowed 1 DSM=100.0025SFI+1 DSM=40,000 SFI). Correlations were sought between the evolution of the graft based on clinical criteria and immunological studies (anti-HLA antibodies by Luminex and Eplets compatibility by HLA-Matchmaker).

Results: 6 patients lost the graft (PRA>90%). 3 patients presented humoral rejection, two of them with graft loss (4.9%). A correlation was observed between the number of incompatible donor-recipient eplets and the anti-HLA antibodies titre (R=0.37). Patients who received plasma exchange posttransplantation had a decrease in the DSAs titre (p<0.05).

Conclusions: Currently a large number of patients included in transplant kidney lists are hyperimmunized, being necessary to look for transplant protocols for them with reduced possibility of humoral rejection. We state that results may indicate that renal transplantation with DSAs imply an assumable risk. However, studies with larger series of patients and with a longer follow-up period are needed to stablish threshold titres of anti-HLA antibodies. Most hyperimmunized patients (PRA>95%) could receive personalized immunosuppressive protocols post-transplant.
In both groups, a statistically significant negative correlation was found between the indicators of the VC\textsubscript{max} and FVC and the level of cyclosporin A (R=0.69, p<0.0001 and R=−0.4, p<0.001) in the first group and FVC and tacrolimus (R=−0.72, p<0.018) in the second group.

Conclusions. A moderate decrease in the VC\textsubscript{max} values in patients after kidney transplantation in condition of immunosuppressive therapy requires monitoring FER and conducting such patients by nephrologists together with specialists in the pulmonological profile.

**P.C3.03.18**

The role of sphingosine-1-phosphate in vascular permeability

G. C. Wilkins\textsuperscript{1}, S. Ali\textsuperscript{1}, N. S. Sheerin\textsuperscript{1,2}, J. A. Kirby\textsuperscript{1};

1Newcastle University, Newcastle upon Tyne, United Kingdom, 2Freeman Hospital, Newcastle upon Tyne, United Kingdom.

Introduction

Organ transplantation is the preferred treatment for end-stage organ failure. However, to meet the ever increasing demand for donor organs, marginal organs are more frequently accepted. Organ quality can be improved by ex vivo perfusion, which also allows for the directed delivery of therapeutics. The signalling lipid sphingosine-1-phosphate (S1P) binds G protein coupled receptors (S1PR1-S5) to affect the endothelial barrier. This study was designed to determine the potential of perfusion with S1PR agonists/antagonists to enhance the endothelial barrier, thereby reducing organ oedema and leukocyte infiltration.

Methods

Dissimilar test results and confusing data interpretation. Recently, cytolytic flow cytometry crossmatch (cytolytic FC-XM) was developed, to detect lytic alloantibodies with a sensitivity of 100% and specificity above 99%.

Thus, the aim of this study was to compare the laboratory crossmatch outcome with use of different assays. This was a virtual immunological matching of deceased donors with hypothetical recipients. Serum from 22 sensitized patients was crossmatched with surrogate donors and in all cases V-XM was positive at minimum 5000 MFI cut off. The positive CDC-XM result was noted in 41% of patients, while positive FC-XM in 86% and lytic antibodies (cytolytic FC-XM) were confirmed in 27%. There was a moderate correlation for the CDC reaction and cytolytic FC-XM level, both for total B cell enriched lymphocytes pool (CDC-XM) and CD3/CD19 lymphocytes (cytolytic FC-XM). When cut-off value of 7000 MFI of highest DSA was used all the positive CDC-XM cases were identified. Similarly, positive FC-XM was followed by 2500 MFI cut-off value. Our results suggest that donor-recipient immunological matching for kidney transplantation requires different methods to verify the importance of alloantibodies. Thus, there is still space for improvement. This is especially important for immunized patients for successful transplantation.

**P.C3.03.19**

**IMMUNE CROSSMATCH FOR KIDNEY TRANSPANTATION - IS THERE SPACE FOR METHOD IMPROVEMENT?**

M. Zielinski\textsuperscript{1}, G. Moszkowska\textsuperscript{2}, H. Zielinska\textsuperscript{1}, A. Dukat-Mazurek\textsuperscript{1}, J. Dębska-Zielinska\textsuperscript{1}, J. Sekowska\textsuperscript{1}, B. Rutkowski\textsuperscript{1}, A. Dębska-Szuleń\textsuperscript{1}, P. Trzonkowski\textsuperscript{1};

1Department of Clinical Immunology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland, 2Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdańsk, Gdańsk, Poland.

There are different laboratory methods for donor-recipient immunological matching before kidney transplantation. Although complement dependent crossmatch is still a gold standard other method have been commonly used like virtual crossmatch (V-XM), and flow cytometry crossmatch (FC-XM). The methods differ in sensitivity and specificity, that may result in dissimilar test results and confusing data interpretation. Recently, cytolytic flow cytometry crossmatch (cytolytic FC-XM) was developed, to detect lytic alloantibodies with a sensitivity of 100% and specificity above 99%.

Thus, the aim of this study was to compare the laboratory crossmatch outcome with use of different assays. This was a virtual immunological matching of deceased donors with hypothetical recipients. Serum from 22 sensitized patients was crossmatched with surrogate donors and in all cases V-XM was positive at minimum 5000 MFI cut off. The positive CDC-XM result was noted in 41% of patients, while positive FC-XM in 86% and lytic antibodies (cytolytic FC-XM) were confirmed in 27%. There was a moderate correlation for the CDC reaction and cytolytic FC-XM level, both for total B cell enriched lymphocytes pool (CDC-XM) and CD3/CD19 lymphocytes (cytolytic FC-XM). When cut-off value of 7000 MFI of highest DSA was used all the positive CDC-XM cases were identified. Similarly, positive FC-XM was followed by 2500 MFI cut-off value. Our results suggest that donor-recipient immunological matching for kidney transplantation requires different methods to verify the importance of alloantibodies. Thus, there is still space for improvement. This is especially important for immunized patients for successful transplantation.

**P.C3.03.20**

**Lymphocytes B as a potential marker of alloantibodies development after kidney transplantation**

M. Zielinski\textsuperscript{1}, A. Larasiewicz\textsuperscript{1}, H. Zielinska\textsuperscript{1}, M. Janowska\textsuperscript{2}, G. Moszkowska\textsuperscript{1}, J. Sekowska\textsuperscript{1}, B. Rutkowski\textsuperscript{1}, A. Dębska-Szuleń\textsuperscript{1}, P. Trzonkowski\textsuperscript{1};

1Department of Clinical Immunology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland, 2Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdańsk, Gdańsk, Poland.

Immunodiagnostic after kidney transplantation is focused on alloantibodies assessment to confirm humoral rejection episodes. Anti-HLA antibodies development is associated with specific B cells differentiation to long-lived memory cells and molecular signature. Thus, it is interesting whether B cells phenotype can be applied as a marker of humoral immunity activation. This may be beneficial for personalized risk stratification. Low-risk kidney transplant recipients (n=53) were followed-up to 24 months after transplantation for alloantibodies development and signs of organ rejection. Every three months anti-HLA antibodies, naïve/memory B lymphocytes as well as CD5+ B cells phenotype were assessed together with Th1/Th2 and BAF7 serum levels. For the 34 recipients, alloantibodies were not present after transplantation, while for 19 others DSA/de novo anti HLA were confirmed. The higher rate was observed 20 months after transplantation. Anti-HLA antibodies development was proceeded by increased in BAF7 and INFγamma levels, as well as memory B lymphocytes numbers. It was found, that alloantibodies development was correlated with a number of memory B cells, Rs=0.96 (Spearman rank correlation).

Lymphocytes B phenotype monitoring after kidney transplantation is useful for alloantibodies development and may serve as an additional marker of humoral immunity activation. This may be beneficial for individual risk stratification and tailored immunosuppression protocol development after kidney transplantation. Funding: National Centre for Research and Development, Poland (No. STRATEGMED1/233368/1/NCBR/2014). National Science Centre, Poland (No. NN402420738 and NN402 562440).

**P.C3.04 MHC, Stem Cell Transplantation and Regulation**

**CCR4 and CXCR8 chemokine receptor expression on high suppressive regulatory T cells**

G. Adigbli;

Transplantation Research Immunology Group, Oxford, United Kingdom.

Introduction: Human regulatory T cells (Tregs) are a promising therapy for the safe control of transplant rejection. The chemokine receptors CCR4 and CXCR8 are expressed by subsets of Tregs with unusually high suppressive activity. However, the mechanisms underling this enhanced suppressive activity are not fully understood and yet to be explored in transplantation. The aim of this study is to characterise the suppressive abilities of CCR4\textsuperscript{+} and CCR8\textsuperscript{+} Treg in transplantation and explore the mechanisms underlying them. Methods: Peripheral blood lymphocytes were sorted by expression of CD3/CD19 and CD45RA. Tregs were then treated with expression of CCR4 and CXCR8 surface receptors before being stimulated with anti-CD3/anti-CD28 beads for 14 days and analysed by flow cytometry. Tregs expanded for 14 days with anti-CD3/anti-CD28 beads were sorted by expression of CCR4 and CXCR8 surface receptors and used in an in vitro suppression assay. Intracellular interleukin (IL)-10, interferon (IFN)-gamma and IL-17 were also measured.

Conclusions. A moderate decrease in the VC\textsubscript{max} and FVC and the level of cyclosporin A (R=0.69, p<0.0001 and R=−0.4, p<0.001) in the first group and FVC and tacrolimus (R=−0.72, p<0.018) in the second group.

Conclusions: We hypothesise that CCR4 and CXCR8 expression identifies a highly suppressive subset of Treg, which may enhance Treg therapy and monitoring of transplant rejection.

**P.C3.04.02**

Letrozole and Testosterone Combination Stimulates Bone Marrow Mesenchymal Stem Cell Proliferation Without Altering Their Characteristics

B. Ara, H. Dagdeviren, T. Simsek, G. Yanikkaya Demirel;

Yeditepe University, Istanbul, Turkey.

Introduction: Due to their immunomodulatory properties which are proven by in vitro studies and clinical trials, mesenchymal stem cells (MSCs) are considered as the most promising cellular therapy agents. However, since they present a limited population in bone marrow specimens and they undergo senescence and lose characteristic features as the passage number increases; it is an important issue to increase their proliferative capacity before re-infusion to patients.Method: Bone marrow mononuclear cells were isolated from 11 individuals’ (healthy n=2 patient: 9) samples by eliminating erythrocytes with ammonium chloride lysis solution. Cells were characterized according to their surface markers (CD73/CD90/CD105/CD34*/DD45*) at the 3rd passage.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
POSTER PRESENTATIONS

Two different doses of testosterone or 17-β estradiol (10 nm and 100 nm) and letrazole (1000 nm) with both doses of testosterone were applied for 72 hours. Proliferation rate was evaluated by means of CFSE staining and BrdU cell cycle analysis. The results showed that testosterone, 17-β estradiol and testosteronem combined with letrazole supplements in both doses were stimulating cell proliferation without changing expression levels of CD3, CD90, CD105 and CD44 marker. Conclusion: In this study, we showed that testosterone stimulates proliferation of BM-SCs when its' aromatisation is inhibited with letrazole addition without any effect on surface markers. Gene level changes with these treatments will be explored in further studies.

P.C3.04.03 The presence of atopic dermatitis in heart-transplanted pediatric patients is associated with an immune Th2 polarization J. Lopez-Abente1, M. Carvalho-Oliveira2, E. Valdivia3, Y. Yuzefovich4, R. Schweizer5, B. Petersen1, J. Seissler1, R. Blasczyk1, C. Figueiredo1.
1Institute for Transfusion Medicine, Hannover Medical School, Germany, 2Hannover, Germany, 3I2. Transplantation Laboratory, Clinic for General, Visceral and Transplantation- surgery, Hannover, Germany, 43. Department of Biotechnology, Institute of Farm Animal Genetics, Friedrich-Löffler-Institute, Federal Research Institute for Animal Health, Hannover, Germany, 5Biotechnology Group, Medical School of Munich, Munich, Germany.

Introduction: The incidence of comorbidities, such as atopic dermatitis (AD), has increased in pediatric heart transplantation during the last decades. Treatment remains a significant challenge in transplantation context. Therefore, understanding the immune mechanisms underlying the disease is important to improve the management of this comorbidity. This study assessed whether potential immune alterations associated with regulatory T cells (Treg) and Th1/Th2 imbalance could be related to AD in heart-transplanted children. Materials and Methods: This single-centre, cross-sectional, observational study included 11 AD and 11 non-AD heart-transplanted aged-matched pediatric patients. Peripheral blood samples were obtained, and immune populations were analysed using flow cytometry. Results: We observed that age at transplant was significantly lower in the AD group. In addition, the development of AD after transplant was associated to a higher frequency of IL-4-secreting CD4+ T cells, a decrease in the ratio of INF-γ to IL-4-secreting CD4+ T cells, an increase in the frequencies of differentiated Treg and eosinophilia. Non-AD patients presented a negative correlation between Treg and IL-4 secreting CD+ T-cell frequencies, however, this correlation was lost in AD patients. Conclusion: The loss of the correlation between Treg and IL-4 secreting CD4+ T cells suggested the potential incapacity of Treg to prevent the expansion of Th2 cells, that are crucial players in AD development and thus potential therapeutic targets. Funding: This work was supported by grants from Instituto de Salud Carlos III (ISCIII) co-financed by federal funds (PI15/00011; IC14/00282, P15/00923). J.L-A. is supported by an ISGSM pre-doctoral grant.

P.C3.04.04 Generation of SLA-silenced porcine pancreatic islets to support graft survival after xenogeneic transplantation M. Carvalho-Oliveira1, E. Valdivia2, Y. Yuzefovich1, O. Pogazhayev1, R. Schweizer3, B. Petersen1, J. Seissler1, R. Blasczyk1, C. Figueiredo1.
1Institute for Transfusion Medicine, Hannover Medical School, Germany, 2Hannover, Germany, 3I2. Transplantation Laboratory, Clinic for General, Visceral and Transplantation-surgery, Hannover, Germany, 43. Department of Biotechnology, Institute of Farm Animal Genetics, Friedrich-Löffler-Institute, Federal Research Institute for Animal Health, Hannover, Germany, 5Biotechnology Group, Medical School of Munich, Munich, Germany.

Introduction: The prevalence of diabetes increased over the last decades. Xenotransplantation of porcine pancreatic islets may offer an alternative source to circumvent the potential incapacity of Treg to prevent the expansion of Th2 cells, that are crucial players in AD development and thus potential therapeutic targets. Fundings: This work was supported by grants from Instituto de Salud Carlos III (ISCIII) co-financed by federal funds (PI15/00011; IC14/00282, P15/00923). J.L-A. is supported by an ISGSM pre-doctoral grant.

P.C3.04.05 Deciphering anti-HCMV HLA-E-restricted unconventional CD8 T-cell responses in seropositive HCMV+ hosts B. CHARREAU1, N. Jouand2, C. Bressollette-Bodin1, N. Gérard1, M. Giraf1, P. Gueri1, A. Rodallec, R. Oger1, T. Parrot1, M. Allard1, A. Cesbron-Gautier1, N. Gervais1.
1CRTI UMR1604, Nantes, France, 2CRTI UMR1604 and CRCSNA UMR1232, Nantes, France, 3CRTI and Service de Virologie, CHU de Nantes, Nantes, France, 4CRTI and CHU de Nantes, Nantes, France, 5Institut de Recherche et de Formation sur le Cancer IRF-CNRS, Nantes, France, 6EFS Pays de la Loire, Nantes, France.

Human cytomegalovirus (HCMV) causes severe illness and poor outcome in immunocompromised hosts such as transplant recipients and HIV-infected patients. Cytotoxic CD8+ T-cell responses, in a large cohort (n=144) of kidney transplant recipients and healthy volunteers, and to elucidate determining factors. HLA-E UL40 transcripts were evaluated by real-time PCR and protein levels by flow cytometry and fluorescence microscopy analyses. The effect of SLA class I silencing was evaluated in human NK cell cytotoxicity assays. Results: Fluorescence microscopy analyses indicated a successful transduction of the islets in its 3D-structure. Expression of NanoLuciferase was inhibited with letrazole addition without any effect on surface markers. Gene level changes with these treatments will be explored in further studies.
Most importantly, expanded Tregs from patients maintained their phenotype and suppressive function after in vitro stimulation in the presence of the pro-inflammatory cytokines IL-2 and IFN-γ. In conclusion, CD105+CXCR5+FOXP3+ Tregs from long-term Belatacept-treated patients can be ex vivo expanded without loss of their phenotype and function. These data demonstrate that despite the reported alterations of Tregs from transplanted patients maintained long term with immunosuppressive therapy, it is possible to use Tregs as immunotherapy for induction of allograft tolerance. Supported by CONACYT #272518. EA, AH and SA were recipients of fellowships from CONACyT.

P.C.3.04.08
Implementation of an HLA typing strategy based on Next Generation Sequencing to improve the characterization of umbilical cord blood units
E. Enrich1, E. Campos2, M. Paprocka1, H. Kraśkiewicz1, A. Klimczak1, U. Kozlowska1, P. Czepiel2
1Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
2Medical University, Faculty of Health Science, Department of Physiotherapy, Wroclaw, Poland.

Introduction: Transfusional Medicine Group, Vall d’Hebron Research Institute, Autonomous University of Barcelona (VHIR-UAB), Barcelona, Spain, 1Congenital Coagulopathy Laboratory, Blood and Tissue Bank, Barcelona, Spain, 2Cell Therapy Unit, Blood and Tissue Bank, Barcelona, Spain, 3CIBER of Cardiovascular Diseases (CIBERCV), Madrid, Spain.

The application of Next Generation Sequencing (NGS) in histocompatibility laboratories has allowed large-scale high-resolution HLA typing, improving and enlarging genotypic information of donors’ registries. The objective of this study was to take advantage of an in-house NGS based strategy for HLA-A, -B, -C, -DRB1 and -DQB1 typing, to improve HLA characterization of umbilical cord blood units (CBUs) from Barcelona Cord Bank. Specific primers were designed to amplify exons 2, 3 and 4 for HLA class I genes and exons 2 and 3 for class II genes in a single multiplex PCR per patient, using an in-house previously validated procedure. Concurrent sequencing of 384 samples was carried out in Illumina MiSeq 500-cycle runs and NGSeqene software was used to HLA genotyping. With this approach 6.000 CBUs and 3.000 samples from cord blood donors’ mothers were typed. An average density of 850 Kclusters/mm² and 86% of cluster passing filter was obtained; 83% bases had a Q-score > 20 and optimal coverage was achieved for the correct typing of each sample. Improvements were performed during its implementation, such as the design of specific primers to identify null alleles or its automation, that allow the typing of up to 768 samples per week. Additionally, 30 new alleles were identified and more than half were submitted to IPD-IMGT/HLA Database. In conclusion, the implementation of this NGS strategy has allowed a cost-effective high-resolution HLA genotyping of a significant fraction of our CBUs repository, which will rebind in a more accurate selection for hematopoietic cell transplantation.

P.C.3.04.09
Rare sequence of complications in a pediatric patient after lung transplantation: identification of donor T cells during GvHD, allospecific CTL during acute rejection and CMV-specific CTL after reactivation supports clinical management
C. S. Falk1, N. Schwerck2, C. Müller3, I. Tuderache1, C. Neudorf4, G. Hansen1, A. Haverich5, G. Warnecke6
1MHH, Institute of Transplant Immunology, Hannover, Germany, 2MHH, Department of Pediatric Pneumology, Hannover, Germany, 3MHH, Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover, Germany.

Background: A 17 year old patient with cystic fibrosis underwent bilateral lung transplantation with immunosuppression (IS) of Tacrolimus, MMF, Prednisone. After 3 months, he developed a biopsy proven case of GvHD, which was successfully controlled by MMF withdrawal but followed by acute rejection of the lung. After treatment by steroid pulse, lung function returned to normal but CMV infection occurred despite valganciclovir prophylaxis. Immunoassays were performed to quantify specific T cell responses as indicators of changes in is. Methods: Frequencies of HLA-A32+ donor lymphocytes were determined by FACS. ELISpots were performed to detect allospecific and CMV-specific T cells. HLA-A2/NEU-multimer staining was used to detect CMV-specific CDB8+ CTL. Results: At the peak of skin GvHD, frequencies of HLA-A32+ donor CD4+CD8+T cells (4%), B (8%) and NK (4%) cells were detected in peripheral blood. After the resolution of GvHD acute, simultaneous improvement of GvHD, acute, mixed leukocyte reaction (MLR) was used to follow the donor T cell response. The O-A32-specific CDB8+ CTL, which declined after steroid pulse. HLA-A11-restricted, GvHD-mediating CTL were detected at low frequency. With serological detection of CMV, HLA-A2/NEU-specific CDB8+ CTL emerged, expanded clonally and persisted for four months. CMV infection disappeared with the appearance of CMV-specific CDB4+ T cells. Conclusions: Using specific immunomonitoring tools, we could follow the clinical course “online” starting with GvHD, followed by rejection and CMV infection. Frequencies of donor lymphocytes, allo- and CMV-specific T cells were indicative for disease stage and adjustment of IS. With full recovery of his lung function, the patient is asymptomatic several months after these complications.

P.C.3.04.10
The development of novel multi-platform flow cytometry parameters to identify immune cells in remote transplant recipients undergoing defined exercise program
G. J. Fatania1, J. E. Pearl2, R. S. Billany3, N. C. Bishop4, A. C. Smith5, A. M. Cooper6
1Department of Infection, Immunity and Inflammation, Leicester Kidney Lifestyle Team, University of Leicester, Leicester, United Kingdom, 2John Walls Renal Unit, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom, 3School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom.

Introduction: Renal transplantation improves renal function but do not reduce cardiovascular risk associated with renal disease. Exercise can reduce both conventional and renal-associated cardiovascular risk; however, exercise can positively and negatively affect immune function depending on the nature of the exercise. It is therefore critical to monitor changes in immune cell populations during exercise. To determine the impact of exercise on circulating immune cells of renal transplant recipients we have developed multi-colour flow cytometry panels covering lymphoid and myeloid populations. Methods: A 10-colour lymphocyte panel and two 6-colour myeloid panels (monocytes and dendritic cells) have been designed to assess changes in the frequency of cells within the peripheral blood of renal transplant recipients undergoing defined exercise programmes. Peripheral blood mononuclear cells were isolated and stained with a panel of markers, used to examine migratory properties of Mac2, and a cell forming assay was performed to assess their clonogenic potential. Results: In both ATMSC and BM-MSC CMV 70% biologically active factors were detected out of 120 tested. A number of growth, angiogenic and immunomodulatory factors, including IL-6, IL-8, MCP and GM-CSF was obtained. Nine factors were selected by BMMSC (e.g. GDFN SCF) and one cytokine only by ATMSC (IL-7). In functional assays both ATMSC stimulated HaCaT proliferation, clonogenic potential and in vitro wound closure compared to control. The optimal effect was observed following a low dose treatment with 25% of MSC-CM. Conclusions: The secretion profile of both lines is similar but not identical. The source of MSC does not significantly influence on MSC-CM’s bioactivity and therapeutic potential on wound repair. These data suggest that MSC-CM is a good source of immunomodulatory and trophic factors and may promote tissue repair. Grant NCN No. 2012/07/B/NZ4/018

P.C.3.04.11
Human adipose-tissue-derived and bone-marrow-derived mesenchymal stem cell-conditioned medium contains immunoregulatory cytokines and exhibit therapeutic potential in wound repair in vitro
S. Gromola1, M. Paprocka, H. Krakiewicz1, A. Klimczak2
1Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
2Medical University, Faculty of Health Science, Department of Physiotherapy, Wroclaw, Poland.

Introduction: Conditioned medium from MSC (MSC-CM) contains mixture of growth and immunoregulatory factors and is perceived as a promising tool for regenerative medicine. We investigated MSC-CMs from two sources: human, immortalized adipose-tissue (ATMSC) and bone-marrow (BM-MSC) for their biological activity for secreted factors and their influence on keratinocyte’s proliferation, clonogenicity and migration. We investigated MSC-CMs from two sources: human, immortalized adipose-tissue-derived (ATMSC) and bone-marrow-derived mesenchymal stem cells (BMMSC) for their biological activity for secreted factors and their influence on keratinocyte’s proliferation, clonogenicity and migration. We investigated MSC-CMs from two sources: human, immortalized adipose-tissue-derived (ATMSC) and bone-marrow-derived mesenchymal stem cells (BMMSC) for their biological activity for secreted factors and their influence on keratinocyte’s proliferation, clonogenicity and migration. We investigated MSC-CMs from two sources: human, immortalized adipose-tissue-derived (ATMSC) and bone-marrow-derived mesenchymal stem cells (BMMSC) for their biological activity for secreted factors and their influence on keratinocyte’s proliferation, clonogenicity and migration.

Materials

Most importantly, expanded Tregs from patients maintained their phenotype and suppressive function after in vitro stimulation in the presence of the pro-inflammatory cytokines IL-2 and IFN-γ. In conclusion, CD105+CXCR5+FOXP3+ Tregs from long-term Belatacept-treated patients can be ex vivo expanded without loss of their phenotype and function. These data demonstrate that despite the reported alterations of Tregs from transplanted patients maintained long term with immunosuppressive therapy, it is possible to use Tregs as immunotherapy for induction of allograft tolerance. Supported by CONACYT #272518. EA, AH and SA were recipients of fellowships from CONACyT.

P.C.3.04.08
Implementation of an HLA typing strategy based on Next Generation Sequencing to improve the characterization of umbilical cord blood units
E. Enrich1, E. Campos2, M. Paprocka1, H. Kraśkiewicz1, A. Klimczak1, U. Kozlowska1, P. Czepiel2
1Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
2Medical University, Faculty of Health Science, Department of Physiotherapy, Wroclaw, Poland.

Introduction: Conditioned medium from MSC (MSC-CM) contains mixture of growth and immunoregulatory factors and is perceived as a promising tool for regenerative medicine. We investigated MSC-CMs from two sources: human, immortalized adipose-tissue-derived (ATMSC) and bone-marrow-derived mesenchymal stem cells (BMMSC) for their biological activity for secreted factors and their influence on keratinocyte’s proliferation, clonogenicity and migration. We investigated MSC-CMs from two sources: human, immortalized adipose-tissue-derived (ATMSC) and bone-marrow-derived mesenchymal stem cells (BMMSC) for their biological activity for secreted factors and their influence on keratinocyte’s proliferation, clonogenicity and migration. We investigated MSC-CMs from two sources: human, immortalized adipose-tissue-derived (ATMSC) and bone-marrow-derived mesenchymal stem cells (BMMSC) for their biological activity for secreted factors and their influence on keratinocyte’s proliferation, clonogenicity and migration. We investigated MSC-CMs from two sources: human, immortalized adipose-tissue-derived (ATMSC) and bone-marrow-derived mesenchymal stem cells (BMMSC) for their biological activity for secreted factors and their influence on keratinocyte’s proliferation, clonogenicity and migration.
POSTER PRESENTATIONS

1. PRE-ACTIVATED MESENCHYMAL STROMAL CELLS INDUCE REGULATORY IMMUNE POPULATIONS IN VIVO AND PROLONG CORNEAL ALLOGRAFT SURVIVAL

K. Lynch\(^1\), O. Treacy\(^1\), X. Chen\(^1\), N. Murphy\(^1\), P. Lohan\(^1\), G. O'Malley\(^2\), M. O'Donnell\(^3\), A. Ryan\(^1\), M. Marcos\(^1\), M. Griffin\(^1\), T. Ritter\(^1\);
\(^1\)Regenerative Medicine Institute (REMEDE), College of Medicine, Nursing & Health Sciences, National University of Ireland, Galway, Galway, Ireland, \(^2\)Discipline of Pharmacology, College of Medicine, Nursing & Health Sciences, National University of Ireland, Galway, Galway, Ireland, and \(^3\)Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary.

Introduction: Chronic allograft rejection remains a major barrier to overcome, with some evidence that a specific subset of antiviral T cells, which also recognise HLA allotypes (i.e. cross-reactive), may contribute to allograft rejection. We have identified a CMV-specific HLA-A*02:01-restricted CD8\(^+\) T cell with a unique TCR signature and common viral infections. Chronic allograft rejection is a major concern in the clinical setting. Our protocol was designed to address the role of specific antiviral T cell subsets in limiting allograft rejection.

Materials and Methods: We used a murine model of corneal transplantation and rejection free survival (RFS) in C57BL/6 mice. We administered MSCs expressing a construction encoding a CMV-specific TCR (CTC) to delay rejection.

Results: MSCs with a CTC were able to delay rejection, and MSCs without a CTC were able to delay rejection to a lesser extent. MSCs with a CTC were able to delay rejection to a greater extent than MSCs without a CTC.

Conclusions: MSCs with a CTC can delay rejection, and MSCs without a CTC can delay rejection to a lesser extent. MSCs with a CTC can delay rejection to a greater extent than MSCs without a CTC.

2. TNF-α-LI ALB144 licensed mesenchymal stromal cells can improve limb functional recovery following sciatic nerve injury.

N. Murphy\(^1\), O. Treacy\(^1\), P. Lohan\(^1\), K. Lynch\(^1\), A. Ryan\(^1\), M. Marcos\(^1\), M. Griffin\(^1\), T. Ritter\(^1\);
REMEDE, NUIG, Galway, Ireland.

Introduction: We have previously demonstrated that allogeneic mesenchymal stromal cells (MSCs) administered intravenously (i.v.) pre-transplantation (day-7, day-11) prolong corneal allograft survival. However, syngeneic (recipient-derived) MSCs administered at these timespoints fail to prolong graft survival. Here, we demonstrate in-vivo that prevention of syngeneic MSCs with pro-inflammatory cytokines TNF-α-LI ALB144 enhances MSC's immunomodulatory properties, potently suppressing syngeneic lymphocyte proliferation (10.26%, p<0.001) compared to untreated MSCs (MSC\(^{\text{null}}\)). Lympocyte suppression was mediated primarily by up-regulation of MSC's nitric oxide production (NO). In vivo, when administered post-transplantation (day-1, day-7) both MSC\(^{\text{null}}\) (50%, p<0.05) and MSC\(^{\text{ALB144}}\) (70%, p<0.001) prolong corneal allograft survival compared to allograft controls (0%). The ability of MSC\(^{\text{ALB144}}\) to prolong graft survival was in part mediated by NO production as mRNA knockdown of NO reduced the graft survival rate of MSC\(^{\text{ALB144}}\) treated animals to 25%. MSC-mediated graft survival was increased with increased proportions of regulatory CD11b/\(^+\)220\(^-\) macrophages and Fopp3/ regulatory T cells (Tregs) in the lung and spleen at day 9 post transplantation while at the time of rejection (day17-19) increased proportions of Tregs were observed in the lung, spleen and crucially the draining lymph nodes of MSC treated allograft recipients. Finally, ex vivo, we report a mechanism of MSC-mediated Treg induction where MSCs in conditions CD11b/\(^+\)220\(^-\) sorted lung cells to a regulatory phenotype which can protect from rejection. This study highlights the importance of timing and licensing strategies in enhancing MSC therapy and sheds light on how MSCs exert their immunomodulatory properties in the lung via a CD11b/\(^+\)220\(^-\) intermediary cell population.

3. TRANSMEM-TSCM.1547087292.77994.1375425012.2230.0561.0.0.N: The number of circulating immature/transitional B cells correlates with the type 3 innate lymphoid cells in hematopoietic stem cell-transplanted patients with acute graft-versus-host disease

Z. I. Komlós\(^1\), J. Z. Pász\(^1\), N. Lugas\(^1\), G. Barna\(^1\), P. P. Reményi\(^1\), G. Tóth\(^1\), G. Király\(^1\), N. Kovács\(^1\), T. Mészáros\(^1\), G. Losonczi\(^1\), C. A. Adács\(^1\);
\(^1\)Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, \(^2\)Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Zurich, Switzerland, \(^3\)Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, \(^4\)Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Zurich, Switzerland, \(^5\)Department of Pulmonology, Semmelweis University, Budapest, Hungary, \(^6\)Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Zurich, Switzerland.

Introduction: The number of circulating immature/transitional B cells correlates with the type 3 innate lymphoid cells (ILC3s) in hematopoietic stem cell-transplanted patients with acute graft-versus-host disease (aGVHD). ILC3s are an intermediary cell population in the pathomechanism of GVHD is controversial. We have recently shown that activated ILC3s can help B cell survival, proliferation, differentiation and the proliferation of immature/transitional B (iT) cells with immune regulatory properties. The impact of the activated ILC3 populations on iT cells after HSCT, and their role in the prevention of GVHD have not been investigated thus far. Therefore, we aimed to examine the interaction of ILC3s and iTB cells in acute GVHD. Twenty patients of hematopoietic stem cell transplantation were included in the study, out of which ten were suffering from acute, aggressive GNHD. Their peripheral blood samples were analyzed before initiation of the treatment. Peripheral blood Lineage CD117/CD161+CD69-ITC cells, and their Nkp44 and CD69 expression, as well as CD191/CD204/CD388 iTB cells and their PD-L1 expression were measured by flow cytometry.
The prevalence of ILC3s was lower in acute GVHD patients compared to HSCT patients without GVHD. Both the absolute numbers and the percentages of ILC3s and iNKT cells co-cultured with each other, and the correlation was particularly strong in acute GVHD patients. Our results suggest that activation of ILC3s may have a favorable influence on the early post-HSCT period via its T-cell-inducing capacity, and iNKT cells with regulatory properties may have a protective role against acute GVHD. Funded by Hungarian Paediatric Oncology Network.

P.C3.04.19
Clonal tracking of CD45RA-depleted donor T-lymphocytes after infusion in TCR alpha/beta-depleted transplantation from unrelated and haploidentical donor
V. E. Fomchenkova1, A. A. Komče1, S. Blagov2, V. Zhagov3, Y. B. Lebedev4, D. M. Chudakov5, M. A. Maschan6, I. V. Zvyagin7;1
1Lomonosov Moscow State University, Moscow, Russia; 2Research Federation, Moscow, Russia; 3Federal Research Center of Pediatric Hematology, Oncology, Immunology, Moscow, Russian Federation; 4Krasnodar Research Center of Pediatric Hematology, Oncology, Immunology, Moscow, Russian Federation.

Conclusions: Low number of T-lymphocytes and extremely low TCR diversity early after alpha-beta T-cell-depleted allogeneic transplantation holds the risk of death from different pathogens, specifically from persisting and reactivating viruses. Transfer of antigen-experienced T-lymphocytes from donor to recipient early after HSCT can provide the protection from pathogens during the period of immunodeficiency. Fracton of CD45RA-depleted T-cells could potentially serve as the source of the antigen-experienced T-lymphocytes and successful implementation of such selective T-cell deple tion was recently shown in the setting of both matched and mismatched HSCT.

Here we report the study of clonal dynamics of CD45RA-depleted donor T-lymphocyte fraction used in low-dose in children after allogeneic HSCT with alpha-beta T-cell depletion. High-throughput sequencing with molecular barcode-based data normalisation was used for TCR beta repertoire profiling of samples of patients’ peripheral blood and CD45RA-depleted donor T-cells (DLC) to track the clonal dynamics of donor cells in patients during 1 year after HSCT.

Small number of both CD8+ and CD4+ T-cell clonotypes from DLI were observed in peripheral blood of patients early after infusion and persisted during 6 months. Their proportion distribution correlates with reconstruction of T-cell repertoire diversity and T-cell count. Most expanded clonotypes in recipients’ repertoire in the early timepoint, including virus-specific T-cell clonotypes, mostly originated from clonotypes which was low frequent or not detected in DLI.

This study was supported by RFBR grant #16-04-01813-a.

P.C4.01.01
Manipulation of tolerance - Part 1

P.C4.01.02
A pro-inflammatory role for CD70 on human regulatory T cells

Arrojo Hornera, K. Wood, F. Isa, J. Hester;
University of Oxford, Oxford, United Kingdom.

Introduction: The CD27-CD70 costimulatory receptor-ligand pair belongs to the TNFR superfamily. Ligation of CD27 on T cells with CD70 on APCs promotes T cell survival, controls CD4+ T cell subset differentiation and it is essential for the generation of antigen-specific T cell immunity. CD70 has also been expressed by T cells upon activation, although the role of the CD27+ T cell-expressed CD70 is unknown. We have previously shown that CD70 expression on human Tregs correlates closely with suppressive potency. In this study, we hypothesised that the CD27/CD70 axis could be exploited for regulating the balance between pro-inflammatory and regulatory T cell responses.

Materials and Methods: Tregs were flow sorted from healthy donors PBMCs and activated for 14 days prior to analysis. Cells were then flow sorted according to CD27 and CD70 and expanded for 14 days prior to analysis.

Results: Human Tregs differently altered CD27 and CD70 surface expression upon activation, resulting in two distinct subpopulations (CD27+CD70+ and CD27-CD70-). Specific functional assays revealed that suppressive activity was confined to CD27+CD70+ Tregs, while CD27-CD70- Tregs showed increased proliferation/expansion capacity, displayed also decreased ability to stimulate proliferation of activated CD8+ T cells and secreted higher levels of IL-17A. Stimulation or blockade of CD27 signalling did not affect Treg suppressive activity. In contrast, blocking CD70 on Tregs significantly enhanced their suppressive potency, abolishing the pro-inflammatory effect of CD70+ T-cells.

Conclusions: This study reveals for the first time that CD70+ Tregs provide stimulatory signals by ligation to CD70 on T cells.

P.C4.01.03
The effects of IFN-gamma on the expansion of suppressive B cells and their action

P. Bohacovaa1, B. Hermankovaa1, Zajicova1,2, B. Hermankova1,2, V. Holana1,2;1Institute of Experimental medicine, Prague, Czech Republic; 2Faculty of Science, Prague, Czech Republic.

Introduction: Besides production of antibodies, B cells can also regulate immune response in the antibody-independent manner. Mechanisms of action of suppressive B cells are mainly the production of IL-10 or the expression of surface inhibitory molecules, such as FasL or PD-L1. In this study we have been aiming to characterize modulatory effects of IFN-γ on the development of suppressive action of B cells.

Materials and Methods: CD19+ B cells were prepared using MACS and stimulated with LPS or LPS and IFN-γ for 48 hours. These cells were harvested and cocultivated with peritoneal macrophages for additional 48 hours. Then macrophage expression of costimulatory molecules was determined by flow cytometry and the cytokine production was evaluated by ELISA. Pretreated macrophages were used for the stimulation of CFSE labeled T cells to measure T cell proliferation by flow cytometry.

Results: B cells stimulated with LPS and IFN-γ produced increased concentrations of IL-10 and also expressed higher levels of the genes for FasL and PD-L1. Macrophages, which were cocultivated with B cells stimulated with LPS and IFN-γ, showed decreased expression of costimulatory molecule CD86 and reduced production of IL-6. These macrophages displayed also decreased ability to stimulate proliferation of activated CD8+ T cells.

Conclusions: The results have shown that IFN-γ enhances activation of suppressive functions of B cells which have the ability to inhibit immune response through their effects on macrophages. The possibility to modulate regulatory B cells may have an impact for their use in a clinical setting.

P.C4.01.04
Early Onset Type 1 diabetes and typical juvenile diabetes are distinct clinical and genetic entities

I. Caramalha1,2, P. Matoso1,2, D. Sabral1,2, J. Costa1,2, D. Ligeiro1, A. Fitas1,2, C. Limbert3, C. Penha-Gonçalves1,2, J. Demengeot3;1Instituto Gulbenkian de Ciência, Oeiras, Portugal, 2Centro de Saúde e Transplantação de Lisboa, Instituto Português de Saúde e Transplantação, Lisboa, Portugal, 3Hospital D. Estefânia, Centro Hospitalar de Lisboa Central, Lisboa, Portugal.

Insulin-dependent Type 1 diabetes (T1D) results from the immune-mediated destruction of insulin-producing pancreatic beta cells, triggered by the interplay between environmental and genetic factors. Susceptibility alleles include Human Leucocyte Antigen (HLA) class II haplotypes and over 100 genetic variants located in more than 50 genetic loci.

In the last decades, an alarming increase in the incidence of T1D affecting preschool children has occurred in Western countries, an unexpected epidemiological concern. We set out to determine whether Early Onset (EO) T1D is clinically and genetically distinct from the typical juvenile T1D. We established a cohort of 100 EO T1D patients (age of onset ≤5 years) and performed their detailed clinical characterization, including familial history of autoimmune diseases. DNA samples were processed for HLA typing, Single Nucleotide Polymorphisms (SNP) genotyping and Whole Exome Sequencing.

P.C4.01.05
Early Onset Type 1 diabetes and typical juvenile diabetes are distinct clinical and genetic entities

D. Sobral1,2, A. Zajicova1,2, B. Hermankova1,2, V. Holana1,2;1Institute of Experimental medicine, Prague, Czech Republic; 2Faculty of Science, Prague, Czech Republic.

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P.C4.01.06
Early Onset Type 1 diabetes and typical juvenile diabetes are distinct clinical and genetic entities

I. Caramalha1,2, P. Matoso1,2, D. Sabral1,2, J. Costa1,2, D. Ligeiro1, A. Fitas1,2, C. Limbert3, C. Penha-Gonçalves1,2, J. Demengeot3;1Instituto Gulbenkian de Ciência, Oeiras, Portugal, 2Centro de Saúde e Transplantação de Lisboa, Instituto Português de Saúde e Transplantação, Lisboa, Portugal, 3Hospital D. Estefânia, Centro Hospitalar de Lisboa Central, Lisboa, Portugal.

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Tolerogenic effects of ethyl pyruvate on dendritic cells

N. Djedovic, M. Mansilla,2 J. Navarro-Barriuso,2,5 S. Stanisavljevic,2 E. Martinez-Cabezés2,2,6, D. Miličković2,2,7
1Federal State Budgetary Institution "Research Institute of Fundamental and Clinical Immunology", Novosibirsk, Russian Federation.
2Instituto Gulbenkian de Ciência, Oeiras, Portugal.
3Institute of Microbiology and Immunology, Belgrade, Serbia.

Introduction: Tolerogenic dendritic cells (DCs) are professional antigen presenting cells that have a key role in shaping the innate immune response. Tolerogenic DC (tolDC) have immune-regulatory properties and they are a promising therapeutic strategy for multiple sclerosis (MS). Ethyl pyruvate (EP) is a redox analogue of dimethyl fumarate (Tecfidera), a drug for MS treatment. We have recently shown that EP ameliorates experimental autoimmune encephalomyelitis (EAE), a MS animal model, and that it induces tolerogenicity in mice. Here we conducted our study on human DC.

Methods: Monocyte-derived DC are obtained from MS patients and healthy individuals in the presence of GM-CSF and IL-4 for 6 days. EP is applied to the cultures on days 2 and 4, while maturation stimulus (TNF, IL-1β, PGE2) is added on day 4 of cultivation.

Results: Phenotypic analysis has shown that DC treated with EP (tEPDC) have significantly reduced levels of molecules required for T cell activation such as CD86, CD83 and HLA-DR whereby CD11c expression and viability of DC were not affected. Further, tEPDC restrained proliferation and modulated cytokine production of allogeneic lymphocytes.

Conclusion: These results demonstrate that ethyl pyruvate has the ability to direct human DC towards tolDC. In vivo study on application of tEPDC in EAE and detailed molecular characterisation of these cells are warranted. These steps should complete pre-clinical studies on tEPDC as potential MS therapy. Funding: MNTRP Republic of Serbia, O17303/1173035. Project Pi14/01175, Pi17/01521, integrated in the Plan Nacional de I+D+I and co-supported by the ISCIII-Subdirección General de Evaluación and the FEDER.

Lipid-polymer hybrid nanoparticles loaded with anti-TNF siRNA suppress inflammation after intra-articular administration in a murine experimental arthritis model

M. A. A. Jansen1, L. H. Klausen2,3, N. Djedovic4,5,6, M. Mansilla2, J. Navarro-Barriuso2,3,1
1Ministry of Education, Science and Technological Development, Republic of Serbia (173035, 173013, 175038).
2Department of Social Medicine, Center of Health Sciences, Federal University of Espírito Santo, Vitória, Brazil.
3Instituto de Medicina Molecular, Lisboa, Portugal.
4Instituto Gulbenkian de Ciência, Oeiras, Portugal.
5Post-Graduate Program in Infectious Diseases, Federal University of Espirito Santo, Vitória, Brazil.
6Centre de Investigación Biomédica de Huelva, Huelva, Spain.

Introduction: Rheumatoid arthritis (RA) is an autoimmune disease which is characterized by chronic inflammation in the joint. RNA interference (RNAi) therapy is a promising way to target gene silencing locally and suppress excessive inflammation. However, for efficient delivery of small interfering RNA (siRNA) into a cell is a problematic. Therefore, there is a need for new and reliable delivery systems. Recently lipid-polymer hybrid nanoparticles (LPNs) for nucleic acid delivery are developed, since these LPNs are less immunogenic compared to other nanoparticles.

Methods: We studied the siRNA delivery of the lipid-polymer-modified poly(DL-lactic-co-glycolic acid) LPNs and stable nucleic acid lipidoid particles (SNALPs) in the murine macrophage cell line RAW 264.7, and investigated their structure-function relationship. Furthermore, we tested the therapeutic capacities of these nanoparticles containing anti-tumor necrosis factor (TNF) siRNA in a murine arthritis model.

Results: Indicate pathway-specific differences in delivery of siRNA to macrophages between LPNs and SNALPs. Both particles were taken up by micropinocytosis but the therapeutic effect of LDNA-SNALPs might have been attenuated by non-specific binding to the cell membrane.

Conclusion: The results from this study show that functional anti-TNF siRNA encapsulated by LPNs or SNALPs can be delivered both in vitro and in vivo. The therapeutic effectivity from LPNs and SNALPs containing anti-TNF siRNA indicates that this is a promising therapy for rheumatoid arthritis and possibly other chronic inflammatory diseases.
Inhibitory oligodeoxynucleotides induce an alternative activation state in human plasmacytoid dendritic cells

P.C4.01.11
Role of NK cells in the onset of Rheumatoid Arthritis.

S. Pascual-García, J. Gallego-Valle, V. Perez-Fernandez, R. Carrea-Rocha, M. Plan;
Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain.

Introduction: Regulatory B cells (Breg) participate in the auto-tolerance maintenance and immune homeostasis. Despite their impact on many diseases and due to the difficulty to define them, knowledge about their origin and their physiological inducers are still unclear. The incomplete knowledge about the generation of Breg and their limited number in primary cultures prevents the development of Breg-based therapy. Therefore, identifying factors that promote their development would allow ex vivo production of large amounts of Breg in order to create new immunotherapies. Materials and Methods: We tested the capacity of several cytokines (IL-1β, GM-CSF and CD40L) and bacteria-derided oligodeoxynucleotides (CpG-ODN), alone or in combination, to generate B cells with regulatory phenotype and function in an in vitro model. By flow cytometry we followed Breg-like phenotype in human primary stimulated B cells.Furthermore, by co-culture experiments we followed suppressive activity of such cells to suppress PBMC proliferation. Results: We have demonstrated that the Breg-associated phenotypes were heterogeneous between one to another stimulation conditions. However, the expression of other markers related to Breg was increased such as IL-10, CD80, CD86, CD71, PD-1 and PD-L1 when cells were stimulated with CpG alone or in combination. Moreover, stimulated B cells presented a suppressive function on autologous activated B cell proliferation. Conclusions: This work demonstrated the feasibility to induce functional Breg-like cells in vitro and then open the way to produce Breg-like as a potential future cellular therapy.

P.C4.01.12
Generation of human Breg-like phenotype with regulatory function in vitro with bacteria-derided oligodeoxynucleotides

J. Gallego-Valle, V. Perez-Fernandez, R. Carrea-Rocha, M. Plan;
Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain.

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P.C4.01.13
Artificial dendritic cells are capable of reprogram effector CD4+ T cells responses from rheumatoid arthritis patients

1Laboratorio de Enfermedades Autoinmunes e Inmunitarias, Programa Disciplinar de Inmunología, Santiago, Chile; 2Unidad de Tratamiento del Dolor, Hospital Clínico, Universidad de Chile, Santiago, Chile, Hospital Del Salvador, Santiago, Chile.

Introduction: Rheumatoid arthritis (RA) is a disabling autoimmune disease generating joint inflammation. A therapeutic approach is the administration of tolerogenic dendritic cells (tolDCs), able to modulate autoantigenic T cell responses and re-establish self-tolerance. The selection of appropriate autoantigens to be presented by tolDC is a critical element in the design of tolDC-based therapies. We aimed to identify immunodominant autoantigen peptides and elucidate the mechanisms by which tolDCs challenged with these self peptides modulate lymphocyte phenotype. Methods: Peripheral blood mononuclear cells (PBMCs) were challenged with 12 autoantigen peptide candidates and reactivity of CD4+ T cells was evaluated based on expression of CD25, CD69, IFN-γ and TNF-α. Peptide-pulsed tolDCs generated from RA patient-derived monocytes were cocultured with autologous tolDCs or peptide-specific T cell lines. Modulation of T cell phenotype and cytokine profile was assessed by flow cytometry. Results: From 12 peptide candidates, recently isolated by our group through natural processing of synovial proteins, we identified 6 immunodominant peptide candidates, derived from calreticulin, vimentin, cofilin, fibrinogen, which generated inflammatory CD4+ T cell responses in 27-39% of RA patients. While mature DCs, pulsed with these autoantigen peptides, induced CD4+ T cell proliferation and pro-inflammatory cytokine production in cocultures, autoantigen peptide-loaded tolDCs were able to modulate this response, promoting hyporesponsiveness of autoactive T cells. Conclusion: We identified 6 novel immunodominant self peptides which are recognized by autoantigenic T cells from RA patients and are therefore appropriate autoantigens for loading of tolDCs used as cell-based therapy of RA.

P.C4.01.14
Inhibitory oligodeoxynucleotides induce an alternative activation state in human plasmacytoid dendritic cells

J. Ruben, S. van der Kooij, C. van Kooten;
Div. of Nephrology and Transplant Medicine, Dept. of Medicine, Leiden University Medical Center, Leiden, Netherlands.

Plasmacytoid dendritic cells (pDC) recognize CpG oligodeoxynucleotides (ODN) via TLR9, leading to the production of type I interferons (IFNα) and enhanced antigen presenting cell functions. Mammalian telomeres and commensal bacteria contain sequences which have been described as inhibitory ODN (iODN), since they act as potent antagonists of TLR9 activation. As such, iODN treatment of animal (autoimmune) models was shown to induce immune regulation. Here, we confirm that iODN can dose-dependently inhibit IFNα production by human pDC, following TLR9 ligation by either CpG or Cytosine-guanosine. In contrast to the IFNα production, TLR9-induced phosphatocytosis could not be inhibited by iODN. In fact, iODN treatment induced phosphatocytosis by pDC in the absence of TLR9 agonists, suggestive for the induction of active signaling. Although pDC treatment with iODN did not induce type I interferons, we showed that iODN treatment of pDCs induced phosphatocytosis by pDCs. In conclusion, we show that iODN directly impact pDC function and should be classified as a group of novel pDC activating ligands, capable of inducing an alternative activation state as compared to CpG ODN.

P.C4.01.10
Inhibitory oligodeoxynucleotides induce an alternative activation state in human plasmacytoid dendritic cells

K. Nazimek1, E. Bustos-Mordard, N. Blas-Rus, P. W. Asknes1, F. Sánchez-Madrid2, K. Bryniarski3;
1Department of Immunology, Jagiellonian University Medical College, Krakow, Poland; 2Department of Immunology, Hospital de la Princesa, Autonomous University of Madrid, Madrid, Spain; 3Section of Allergy and Clinical Immunology, University School of Medicine, New Haven, United States.

Introduction. Mouse contact hypersensitivity reaction (CHS) is suppressed by T CD8+ cell-derived miRNA-150 carried by exosomes coated with antigen-specific antibody light chains (J Allergy Clin Immunol 2013;132:170-81). These exosomes target antigen-presenting macrophages that in turn suppress CHS effector cells [Immunology 2015;146:23-32]. Non-CpG-mediated interactions between these exosomes and iODN-activated pDC were recently examined, showing the passage of CD63-positive exosomes from intact T to B lymphocytes forming the conjugates (immune synapses) [Nat Commun 2011:2:282]. Our current study aimed at investigating the exosomes carrying miRNA-150 on the vesicle-dependent intercellular interactions at the immune synapse. Methodology. Jurkat T cells and Raji B cells were transfected with CD63-GFP or CD81-GFP plasmids by electroporation and then incubated with miRNA-150-containing exosomes. Supernatant-stimulated formation of conjugates by Jurkat T cells and Raji B cells was then assessed in fluorescenceconfocal microscopy and intercellular transmission of vesicles as well as T cell activation was analyzed cytochemically. Results. The polarization of Raji B-cell, CD63-GFP-positive multivesicular bodies towards the side of CD3 accumulation in T (immune synapse) was observed. Further, miRNA-150-carrying exosomes induced transfer of CD81-positive, but not CD63-positive, vesicles from Raji B cells to Jurkat T cells. Afterwards, Jurkat T cells were characterized by lower CD63 expression and increased and aberrant expression of the IgG Fc receptors. The author has a grant supported by Ministerio de Educación, Cultura y Deporte with reference FPU14/01984.
POSTER PRESENTATIONS

P.C4.01.15
V. Tereshchenko, J. Khantakova, V. Kurlin, A. Silkyu, J. Schewecken, J. Lapontikova, A. Maksyutov, S. Sennikov;
Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

Introduction: CD4+CD25+Foxp3+ T-cell receptors are necessary for establishment and maintenance of immune tolerance. We explored the capacity of tolerogenic dendritic cells (tolDCs) transfected by DNA plasmid encoding H-2K epitopes to induce functional Treg cells for tolerance induction.

Materials and methods: DCs were generated from C57Bl/6 mice bone marrow monocyte pool with addition of rmGM-CSF and rmIL-4 without maturation factors. DCs were electroporated with different plasmids: pGEM-H2-Kb and pGEM in the locus epitopes of CBA mice (DCs-pGEMH2-Kb) and control plasmid (DCs-pGEM). Electroporated DCs were cultured with autologous lymphocytes to generate functional Tregs. The phenotype properties of the DCs (CD11c, H2, CD80, CD86, CD83, and CD40) and Tregs (CD4, CD25, FoxP3, and IL-10) were investigated using flow cytometry. Functional capacity of transfected DCs and Tregs to induce tolerance was investigated by mixed lymphocyte reaction.

Results: DCs-pGEMH2-Kb represent a tolerogenic phenotype (increase H2-B and decrease CD80, CD86) compared to DCs-pGEM. DCs-pGEM induce FoxP3 and IL-10 expression by IL-2 and IL-15 stimulation. Cross-talk between the maternal endometrial micro-environment and tolerogenic dendritic cells (tolDCs) transfected by DNA plasmid encoding H-2K epitopes of CBA mice to generate functional Treg cells for tolerance induction.

Conclusion: DCs transfected by DNA plasmid encoding H-2K epitopes of CBA mice induce functional Treg cells in splenocytes cultures and guide tolerance in vitro. The research carried out with the support of the RSF. Agreement №16-15-00086 (11.01.2016).

P.C4.01.16
Hemoglobin is preferentially inflammatory, antigenic and immunogenic in lupus
1National Institute of Immunology, Delhi, India.

Hemolysis-associated anemia is characteristic of diseases such as atherosclerosis, lupus, malaria, and leishmaniasis; the toxic effects of free hemoglobin (Hb) have been extensively described. This study was based on the premise that release of this sequestered, inflammatory molecule can result in deleterious immunological consequences, particularly in the context of pre-existing lupus. PBMcs derived from SLE patients preferentially secreted a variety of immunostimulatory cytokines in response to Hb, and IgG anti-Hb responses were detected in the sera of lupus patients. Lupus-prone mice exhibited heightened plasma Hb levels, and Hb triggered the preferential release of lupus-associated cytokines from splenocytes derived from aging mice. Additionally, Hb induced the release of IL-17A, IL-12 and IL-8 from plasmacytoid dendritic cells, while also eliciting the release of a spectrum of inflammatory cytokines from purified CD8 T cells. CD4 T cells and B cells derived from such mice. Anti-Hb B cell precursor frequencies were heightened in lupus-prone mice, which exhibited increased titers of anti-Hb antibodies in serum and in kidney eluates. Hb interacted with lupus-associated autoantigens extruded during apoptosis, and co-precipitation of Hb and autoantibodies had maturation-inducing effects on bone marrow-derived dendritic cells from lupus-prone mice. Immunization of such mice with Hb induced antigen spreading to lupus-associated moieties; increased complement deposition in the kidneys and enhanced-onset glomerulosclerosis were observed. Hb therefore elicits increased inflammatory responses from a variety of cell types, demonstrates both antigenicity and immunogenicity, and triggers specific immunopathological effects in a lupus milieu.

P.C4.01.17
Treatment of collagen-induced arthritis mice model with genetically modified tolerogenic dendritic cells
I. Yilmaz, M. Karacay, G. Guvenç, E. Üs, F. Budak, F. Ersoy, M. Yalçın, H. B. Oral;
1Department of Medical Immunology, Institute of Health Sciences, Uludag University, Bursa, Turkey, 2Department of Veterinary Pathology, Institute of Health Sciences, Uludag University, Bursa, Turkey, 3Department of Molecular Biology & Genetics, Faculty of Arts & Sciences, Uludag University, Bursa, Turkey, 4Department of Immunology, Faculty of Medicine, Uludag University, Bursa, Turkey, 5Department of Physiology, Faculty of Veterinary, Uludag University, Bursa, Turkey.

T cells activation has an important role in RA pathogenesis. Activation of T lymphocytes requires the co-stimulatory signals provided by antigen-presenting cells. In this study, to inhibit the activation of T lymphocytes in experimental arthritis, tolerogenic dendritic cells (tolDCs) were aimed to be obtained by the genetic modification of bone marrow-derived dendritic cells (BM-DCs). B7 co-stimulatory molecules expression were down-regulated with a gene construct encoding a modified cytotoxic T lymphocyte antigen 4 molecule (CTLA-4 KO) which targets to the endoplasmic reticulum (ER). Mouse CTLA-4 DNA mammalian expression plasmid (pcMV/mCTLA4) was commercially provided from Sirio Biologicals (China). The mCTLA4 gene was cloned into pcMV/myc/ER (Invitrogen, Life Technologies, USA). Plasmids subcloned into LeGio-IG2 (AddGene, USA) for lentiviral vector production. BM-DCs were non-viral and lentiviral transfected with CTLA-4-KDEL and incubated for 48 hours. Flow cytometric analysis was performed with mouse monoclonal antibodies against CD80 and CD86 (Tonto Biosciences, United Kingdom) and appropriate isotype controls. Furthermore, in vivo studies, tolDCs were transferred intraarticularly 3 times to collagen-induced arthritis (CIA) mice model and followed up for 4 weeks. It was observed that CD80/86 expression on the surface of BM-DCs significantly downregulated as tolDCs. Moreover, in vivo studies showed that tolDC treatment group significantly reversed the increase in the joint thickness and number of white blood cells compared with control groups. This study is supported by The Scientific and Technical Research Council of Turkey (TUBITAK-COST Project No: 1145354) under Cost Action BM1404. COST is supported by the EU Framework Programme Horizon 2020.

P.C4.02.01
Manipulation of tolerance - Part 2

P.C4.02.02
Cross-talk between the maternal endomterial micro-environment and tolerogenic dendritic cells
G. Amoldi, P. Panina-Bordignon, C. Semino, S. Sennikov;
1San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy, 2Division of Genetics and Cell Biology, IRCCS San Raffaele Scientific Institute, Milan, Italy.

Successful pregnancy involves highly coordinated interactions between decidualized endometrial stromal cells and maternal immune cells to generate a repressive "maternal niche" for embryo implantation. DC-10 are a subset of dendritic cells, expressing HLA-G and secreting IL-10, which modulate T cell responses, promote tolerance, and accumulate in decidua during the first trimester of pregnancy. To assess whether DC-10 are critical components involved in favoring embryo implantation and successful pregnancy, we studied the in vitro crosstalk between endometrial stromal cells and DC-10. Immortalized human endometrial stromal cells (T-HESC) were in vitro decidualized with cAMP and progesterone and prepared for colonization by peripheral blood CD14+ monocytes. As control, DC-10 were differentiated from the same monocytes without T-HESC supernatants. Differentiated DCs were characterized by: i) expression of DC-10-associated markers; ii) cytokine profile; iii) ability to suppress a primary allogeneic response in vitro. Results showed that monocytes cultured with T-HESC-conditioned media collected at day 2 post-hormonal stimulation, differentiated into DC-10-like cells as demonstrated by the expression of CD141, CD141 and CD163, and display a cytokine secretion profile similar to that of DC-10. Moreover, DC-10-like cells suppress allogeneic CD4+ T cell responses showing a functional parallel with DC-10. These results support the hypothesis that the decidua micro-environment of early pregnancy sustains and promotes tolerogenic DC-10 differentiation. We are currently investigating the interactions of DC-10-like cells with the different immune players known to influence pregnancy outcome as NK cells.

P.C4.02.03
Milk protein-specific IgE and IgG4 in pediatric patients with food allergy
C. Kang, Y. Yang, B. Chiang;
Department of Pediatrics, Taipei, Taiwan.

Introduction: Food allergy is gaining attentions from the clinicians as it leads the development of atopic march. Milk proteins are the first foreign proteins an infant could encounter in life. We studied the prevalence and manifestation of milk protein sensitization among pediatrics.

Methods: We retrieved test results of serum allergen-specific tests (Mast and ImmunoCAPS, respectively). Any IgE level of specific food allergen higher then 2+ (range: 0~4+) in in vitro results was considered positive. In addition, milk protein-specific IgE and IgG4 will be also assessed.

Results: Total 1064 pediatric patients (55.6% of total 1914 patients) had Mast test, while 196 pediatric patients (6.61% of total 2966 patients) received ImmunoCAPs. The percentage positive for food specific IgE were 33.5% in (n = 357) and 20.9% (n = 41) respectively for each tests. The age of milk IgE(+) patients are significantly younger than the general food IgE(+) patients (2.83 ± 1.79 years vs 5.75 ± 3.92 years, p-value 0.005). Most of the milk IgE(+) patients presents with atopic dermatitis clinically, especially patients with higher titers.

Conclusions: The prevalence of milk protein sensitization was lower than what we expected, with the majority among children less than 5 years old. The age of milk IgE(+) patients is significantly younger than patients with other kinds of food IgE. The development of tolerance to milk protein over time might play a role in the reduction of milk protein-specific IgE.
P.C4.02.04
Opioid analgesics differently modulate immune responses in mice
I. Filipczak-Bryniarska1, K. Nazimek2, M. Kazlowski1, M. Wasik1, K. Bryniarski2
1Department of Pain Treatment and Palliative Care, Jagiellonian University Medical College, Krakow, Poland, 2Department of Immunology, Jagiellonian University Medical College, Krakow, Poland.

Introduction. Immune cells commonly express opioid receptors and thus could be stimulated with opioids exerting immunomodulatory effects. Current research aimed at investigating the influence of various opioid analgesics on immune responses in mice under homeostatic conditions.

Methods. Mice were treated intraperitoneally with respective opioid drug (morphine, fentanyl, methadone, buprenorphine, oxycodone) for a week and, in some instances, skin-sensitized with hapten to induce contact hypersensitivity (CHS). Macrophage-induced humoral immunity was assessed in plaque-forming assay together with measurement of antibody titers in sera of mouse recipients of SRBC-pulsed macrophages from mice treated with different opioids. In addition, the effects of opioids on the production of reactive oxygen intermediates (ROI), nitric oxide and cytokines by peritoneal macrophages along with expression of surface markers were estimated.

Results. Morphine administration significantly intensified CHS response in actively sensitized mice, while buprenorphine or oxycodone administration exerted the opposite effect. All tested opioids enhanced the release of proinflammatory cytokines, ROI and nitric oxide by macrophages and altered the production of antigen phagocytosis and presentation markers. In contrast to buprenorphine administration, morphine, fentanyl and methadone treatment impaired humoral immunity induced by macrophages. However, little is known about the possible impact of opioid drugs on opioid-induced immune effects. Thus, current study aimed at investigating the influence of analgesic opioids on morphine-activated immune effects in mice.

Methods. Mice were treated intraperitoneally with morphine, naloxone, amphetamine, gabapentin and/or venlafaxine for a week. Macrophage-induced humoral immunity was assessed in plaque-forming assay together with measurement of antibody titers in sera of mouse recipients of SRBC-pulsed macrophages from mice treated with different opioids. In addition, macrophage production of ROI, nitric oxide and cytokines along with expression of surface markers were estimated.

Results. We observed an overall decrease in cytokine, ROI and nitric oxide production by macrophages from opioid-treated mice, with the strongest effect of amphetamine administration. Further, addition of opioids amplified morphine-induced inhibition of humoral immune response activated by macrophages pulsed with SRBC.

Conclusions. Our current study confirms that macrophages greatly contribute to immunomodulatory effects induced by opioids. Better understanding of mechanisms of immunomodulation by opioids has great importance allowing for evaluation of its beneficial and adverse effects on patient condition.

P.C4.02.05
Immune effects of opioid drugs are modulated by analgesic adjuvants
M. Kazlowski1, I. Filipczak-Bryniarska1, K. Nazimek2, M. Wasik1, K. Bryniarski2
1Department of Pain Treatment and Palliative Care, Jagiellonian University Medical College, Krakow, Poland, 2Department of Immunology, Jagiellonian University Medical College, Krakow, Poland.

Introduction. Opioids exert immunomodulatory effects. We have shown that repeated administration of morphine increases cell-mediated allergic response in mice and, as fentanyl, methadone, buprenorphine and oxycodone, enhances the release of proinflammatory cytokines, reactive oxygen intermediates (ROI) and nitric oxide by macrophages and alters the production of antigen phagocytosis and presentation markers. In contrast to buprenorphine administration, morphine, fentanyl and methadone treatment impaired humoral immunity induced by macrophages. However, little is known about the possible impact of opioid drugs on opioid-induced immune effects. Thus, current study aimed at investigating the influence of analgesic opioids on morphine-activated immune effects in mice.

Methods. Mice were treated intraperitoneally with morphine, naloxone, amphetamine, gabapentin and/or venlafaxine for a week. Macrophage-induced humoral immunity was assessed in plaque-forming assay together with measurement of antibody titers in sera of mouse recipients of SRBC-pulsed macrophages from mice treated with different opioids. In addition, macrophage production of ROI, nitric oxide and cytokines along with expression of surface markers were estimated.

Results. We observed an overall decrease in cytokine, ROI and nitric oxide production by macrophages from opioid-treated mice, with the strongest effect of amphetamine administration. Further, addition of opioids amplified morphine-induced inhibition of humoral immune response activated by macrophages pulsed with SRBC.

Conclusions. Our current study confirms that macrophages greatly contribute to immunomodulatory effects induced by opioids. Better understanding of mechanisms of immunomodulation by opioids has great importance allowing for evaluation of its beneficial and adverse effects on patient condition.

P.C4.02.06
Lymph node stromal cells control T follicular helper cells as well as cell responses directed against a self-antigen
R. Nadafi1, E. D. Keuning1, M. N. Erkenlis1, A. Bos1, M. van Goor2, A. Breedeweld3, R. M. Reijners3, L. G. M. van Baarlen4, R. E. Mebius1
1Molecular cell biology and immunology, VU university medical center, Amsterdam, Netherlands, 2Department of hematology, Leiden university medical center, Amsterdam, Netherlands, 3Department of Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands, 4Department of Rheumatology & Immunology, Academic Medical Center (ARC), Amsterdam, Netherlands, 5Academic Medical Center, Amsterdam, arthriticis, the production of autoantibodies by B cells against self-antigens. To have an effective germinal center response and antibody production, B cells cooperate with T follicular helper cells (Tfh) to undergo somatic hypermutation and improve the affinity of antigen recognition. Tfh formation and differentiation occurs in the T cell area of the lymph node where a highly organized network of lymph node stromal cells (LNSCs) control and regulate peripheral immunity. Here, we use in vivo murine lymph node transplantation model and show that LNSCs can clearly repress formation of autoreactive Tfh while maintaining T regulatory cells (Treg) specific for a given self-antigen. Moreover, control of Tfh formation by LNSCs significantly reduced the germinal center B cells response directed against self-antigen. Importantly, inhibition of IL-2 reduced the LNSC-mediated maintenance of Tregs and released the repression of autoreactive Tfh cell formation in vivo. These findings show that continuous presentation of self-antigens by LNSCs plays a critical role for Treg maintenance while repressing of Tfh and germinal center B cell formation directed against these self-antigens. Importantly, inhibition of IL-2 reduced the LNSC-mediated maintenance of Tregs and released the repression of autoreactive Tfh cell formation in vivo. These findings show that continuous presentation of self-antigens by LNSCs plays a critical role for Treg maintenance while repressing of Tfh and germinal center B cell formation directed against these self-antigens. Ultimately, these findings provide opportunities to modulate humoral immunity at different stages of rheumatoid arthritis.

P.C4.02.07
Intravenous administration of antigen-coupled red blood cells induces suppressor T CD8+ cell recurrent release of suppressive antibodies against self-antigens
K. Bryniarski1, K. Nazimek1, M. Wasik1, M. Ptak1, W. Ptasz1, P. W. Askenase2
1Department of Immunology, Jagiellonian University Medical College, Krakow, Poland, 2Section of Allergy and Clinical Immunology, Yale University School of Medicine, New Haven, United States.

Introduction. Mouse contact (CHS) and delayed-type hypersensitivity (DTH) responses are suppressed by intravenous administration of syngeneic red blood cells (RBC) coupled with protein or antigen, respectively. Current studies aimed at investigating the mechanism of RBC-induced suppression.

Methods. CBA/J, C57BL/6 or BALB/c mice were injected intravenously with trinitrophenyl or ovaloxalone hapten-coupled RBC or with ovaloximun-conjugated RBC and then were either intraperitoneal with hapten or intradermally immunized with ovaloximun. In some instances, tolerized mice were either injected intraperitoneal with clodronate liposomes or immunized second time after DTH elicitation. Supernant of tolerized mouse lymph node and spleen cell culture was filtrated and ultracentrifuged.3 (100.000g) and pelleted exosomes were tested for their suppressive activity in adoptively transferred CHS or DTH. In some cases, cells prior to culture were positively or negatively selected according to CD3, CD4 and CD8 expression.

Results. Intravenous injections of hapten or antigen-coupled RBC induced the release of suppressive, mIgA-carrying exosomes by T CD8+ cells, that were preliminarily shown to mediate the memory of suppression. Further, suppressive exosomes targeted antigen-presenting macrophages that in turn inhibited CHS or DTH effector T cells, while mIgA-150kD mice failed to produce suppressive exosomes.

Conclusions. Our current results suggest that adjuvants normalize morphine-increased macrophage innate activity, which seems to have great importance during sterile inflammation. However, adjuvants additionally impair B cell activation that is reduced by morphine treatment.
POSTER PRESENTATIONS

P.C4.02.08
Repeatability administration of hypotensive drugs and diuretics shifts immunity towards Th2-type in healthy mice
P. Bryniarski1, S. Strobel1, A. Chmielowski2, M. Michalak1, K. Bryniarski1, K. Nazimek1
1Department of Immunology, Jagiellonian University Medical College, Krakow, Poland; 2Students’ Scientific Society, Department of Immunology, Jagiellonian University Medical College, Krakow, Poland.

Introduction. Nowadays, altered inflammatory reactivity of immune cells, especially those infiltrating perivascular tissues, is associated with pathogenesis of hypertension.

However, little is known about possible immunomodulatory effects of clinically relevant hypotensives and diuretics. Therefore, our current studies aimed to investigate the effect of these drugs on immunity in healthy mice.

Methodology. 10-week-old CBA mice were treated intraperitoneally with the following drugs: propanolol, hydrochlorothiazide (10mg/kg), carvedilol, captopril, verapamil, furosemide (5mg/kg), amiodipine (3mg/kg) or olmesartan (1mg/kg) for 7 days. On the third day of drug administration, mice were either sensitized with hapten or intraperitoneally injected with injected oil. Five days later mice were either challenged with hapten to elicit sensitivity (CS), or peritoneal macrophages were collected for assessment of their phenotypic, cytokine production or for humoral immunity testing, after pulsing with SRBC.

Results. Amlodipine administration slightly increased generation of oxygen and nitrogen radicals and all drugs’ administration caused decreased secretion of pro-inflammatory cytokines and slightly enhanced production of anti-inflammatory cytokines by macrophages, and furosemide increased this effect. SRBC-pulsed macrophages from drug-treated mice more potently activated splenic B cells to release antigen-specific antibodies. Finally, all tested drugs, amiodipine and verapamil especially, at both, the induction and effector phase of Th2, suppressed cellular immune response.

Conclusion: Our research findings showed that hypotensive modulate immunity by affecting macrophage function and by polarizing towards Th2-type. Further research should be conducted to examine clinical effect of those observations. Supported by K/DSC/00359S and partly by budget funds for science in 2017-2021 under the “Diamond Grant” program (0168/DoA/2017/46).

P.C4.02.09
Inhibiting squamous synthesize increases cellular tolerance to cholesterol-dependent cytolysins
M. Pospiech1, S. Owners2, D. Miller1, R. K. Allemann1, I. M. Sheldon1
1Swansea University Medical School, Swansea, United Kingdom; 2Cardiff University School of Chemistry, Cardiff, United Kingdom.

BACKGROUND: During infection bacteria secrete toxins that damage the epithelium of the skin and mucosa. Whilst antibiotics are commonly used to treat infection, another approach is to improve the host tissues to tolerate pathogens. Cholesterol-dependent cytolysins are secreted by bacteria and target cholesterol-rich areas of mammalian cell plasma membranes, where they form pores, which leads to cell lysis. The present study aims to inhibit squamous synthesize to reduce the biosynthesis of cellular cholesterol and increase cellular tolerance to cholesterol-dependent cytolysins.

METHODS: Novel bishophosphate compounds were designed to inhibit squamous synthesize, and synthesised de novo. The bishophosphonates were screened for their ability to inhibit squamous synthesize in a cell-free system using a radiometric assay. Twenty bisphosphonates were then evaluated by treating HeLa cells prior to a challenge with a concentration of the cholesterol-dependent cytolysin, pyolysin, that causes 90% cytolysis. Cytolysis was evaluated using the MTT assay, and pore formation was evaluated by measuring the leakage of cellular potassium ions and LDH protein.

RESULTS: Reference compounds as zaragozic acid and methyl-β-cyclodextrin treatment of cells reduced the cytolysis caused by pyolysin by 95%. Amongst the 20 bisphosphonates, we identified two lead compounds that reduced cytolysis caused by pyolysin in a concentration-dependent manner, with a maximum reduction of cytolysis of 95% and 81%. These lead bishophosphonates also prevented short-term potassium leakage from cells, and reduced longer-term LDH leakage by 92% from cells challenged with pyolysin. In conclusion, inhibition of squamous synthesize by bishophosphonates increased the ability of HeLa cells to tolerate a cholesterol dependent cytolysin.

P.C4.02.10
Role of Pbx-regulating-protein 1 (Prep1) in the control of effector and regulatory T cell response in metabolic disorders
C. Procaccini1, D. Faichh1, S. Cabaro2, A. Lioi1, F. Oriente1, V. Giganoto1, F. Blasi1, P. Formisano4, E. Beuginot4, G. Matos-Perez2; 1Ist. Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR), Napoli, Italy; 2URG ‘Genomica Funzionale’ Istituto di Endocrinologia ed Oncologia Sperimentale ‘G. Salvatore’, Consiglio Nazionale delle Ricerche (IEOS-CNR), Napoli, Italy; 3Dipartimento di Scienze Mediche Traslazionali, Università degli Studi di Napoli, ‘Federico II’, Napoli, Italy; 4Istituto Nazionale Tumori (IRCCS), Fondazione Pascale, Napoli, Italy; 5IFOM Institute of Molecular Oncology, IFOM-IEO Campus, Milano, Italy.

Background: Recently, Prep1 expression was identified in human T helper type 17 (Th17) cells, and we observed that Prep1 was also expressed in human regulatory T cells (Tregs), but its role in the development and function of Tregs in vivo remains to be elucidated.

Methods: We generated Prep1 conditional knockout (cKO) mice and analysed T cell development and function in vivo. We tested Prep1 cKO mice in the context of metabolic diseases, e.g., obesity, type 2 diabetes and multiple sclerosis (MS), and we compared Prep1 cKO mice with Prep1 conditional overexpression (cOE) mice.

Results: Prep1 cKO mice displayed reduced T cell functionality and reduced FoxP3+ Treg frequency in the thymus and spleen. Prep1 cKO mice were more susceptible to experimental autoimmune diabetes (EAD) and multiple sclerosis (EAE) models. Prep1 cKO mice had increased blood glucose and insulin resistance levels, high levels of pro-inflammatory cytokines (IFN-γ, TNFα) and reduced levels of anti-inflammatory cytokines (IL-10, TGFβ) in the serum and spleen. Prep1 cKO mice had reduced splenomegaly and weight gain compared to Prep1 cOE mice. Prep1 cKO mice had reduced splenic T cell proliferation and reduced lymph node size. Prep1 cKO mice had reduced T cell functionality in vitro. Prep1 cKO mice had reduced T cell functionality in vivo. Prep1 cKO mice had reduced T cell functionality in vivo.

Conclusion: Prep1 is an important regulator of T cell function in metabolic disorders. Prep1 cKO mice are a valuable model to study the role of Prep1 in T cell function and metabolic disorders.

P.C4.02.11
Identification of immune tolerance-related aberrant epigenetic markers in T cells from multiple sclerosis patients
D. Avancini1, V. Martellini1, C. Farina2, S. Gregori2, 3, R. F. Santoni de Sio1; 1San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), San Raffaele Scientific Institute IRCCS, Milan, Italy; 2Institute for Experimental neurology (INSEPE), San Raffaele Scientific Institute IRCCS, Milan, Italy.

The molecular mechanisms underlying the breakage of immune tolerance triggering multiple sclerosis (MS) autoimmune manifestations remain unclear. Recently, deregulation in epigenetic control has been associated with immune cell alterations and MS-related autoimmunity. We have studied epigenome and transcriptome in CD4+ T cells from peripheral blood of relapsing-remitting MS patients and matched healthy controls (HC) to identify aberrant epigenetic hubs (nodes of aberrantly activated regulatory elements and target genes). We identified by ChiP-seq analysis a number of Differentially Active Regulatory Elements (DARE), most of which are active in HC and less/not active in MS cells. We defined DARE-target genes by intersecting the DARE list with public 3C-based datasets. DARE-target genes resulted over-represented among genes up-regulated in the RNA-seq analysis of HC and enriched in autoimmune/inflammatory disease gene ontology classes, confirming the differential activity of DARE in MS and HC T cells and suggesting their functional role in MS autoimmune manifestations.

The molecular network controlling the activity of DARE in MS, we in silico mapped transcription factor (TF) binding sites and found some interacting master TF enriched for regulatory elements in MS, suggesting that Prep1 might represent a novel platform for potential therapeutic intervention in MS.

P.C4.02.12
Regulatory T cell numbers in inflamed skin controlled by ALK3 signaling in dendritic cells
T. Scano1, M. Hochgherner1, I. Borek1, E. Schwarzenberger1, C. Tam-Amersdorfer1, H. Strabl2; 1Otto Loewi Research Center, Chair of Immunology and Pathophysiolog, Graz, Austria; 2Institute of Cancer Research, Medical University of Vienna, Vienna, Austria.

Bone morphogenetic protein 7 (BMP7) is expressed at aberrant high levels by keratinocytes during skin inflammation and induces inflammatory-type Langerhans cell (LC) differentiation. Regulatory T cell (Treg) generation is enhanced in inflamed epidermis and LCs are an essential Treg source. Thus, we asked whether BMP signaling in LCs may promote Treg generation. Human LCs were generated from CD34+ cells in response to either BMP7 or TGF-B1, to generate LCs resembling inflammatory LCs or steady-state-like LCs, respectively. LCs were cultured with allogeneic CFSE-labeled naïve CD4+ T cells. BMP7-LCs displayed an enhanced T cell stimulatory capacity compared to TGF-B1-LCs. Also, the extent of Treg content utilizing BMP7-LCs was significantly higher than that derived from TGF-B1-LCs. Tregs derived from BMP7-LCs were significantly more immunosuppressive than that derived from TGF-B1-LCs. In vivo validation was carried out by measuring FoxP3+ T cell frequencies in DC-specific ALK3/BMP1R1 knockout mice in an imiquimod induced model of skin inflammation. ALK3 deficiency was associated with fewer FoxP3+ cells compared to wild type mice. Subsequently, molecules that interfere with LC-mediated Treg induction were evaluated. An interfering ALK3 fusion protein prevented specifically derived Treg differentiation in an APC-free system from naïve T cells in vitro. These data demonstrate that BMP/ALK3 signaling in inflamed skin enhances Treg numbers at least partially via modulation of DCs.
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P.C4.02.13 Investigation of the molecular mechanisms that affect regulatory T cell function in patients with active systemic lupus erythematosus
C. Albany1, Z. Catak2, D. McCluskey1, G. Gigan1, L. Neuf1, G. A. Powler1, M. Catapano1, M. Robson1, J. Spencer1, D. O'Cuinn3, R. I. Lechler1, G. Lombardi3, C. Scotti3
1King's College London, London, United Kingdom, 2Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom.

Systemic lupus erythematosus (SLE) is a multisystem, chronic, debilitating and frequently-relapsing condition. Although anti-inflammatory treatments are available, none is curative and relapses remain common. There is a critical requirement for effective therapies that obviate the need for immunosuppression and restore self-tolerance. Regulatory T cells (Tregs) in healthy individuals prevent the development of autoimmune diseases; however, in SLE-patients Tregs are numerically deficient and/or functionally impaired. Ex vivo expansion of Tregs brings within reach the opportunity to obtain a clinical product for therapy; an attractive and novel option for re-establishing self-tolerance. Functional, phenotypic and genetic data were collected from SLE-patients either freshly isolated or cultured in the presence of rapamycin. Results showed that although SLE-Tregs resemble the typical phenotype of healthy-Tregs, their suppressive capacity is impaired during active disease. The analysis of Treg sub-populations revealed differences in cell-distribution during the relapsing course of the disease that correlate with the suppression of the suppressive function. However, the ex vivo expansion of Tregs from SLE-patients in the presence of rapamycin fully restored their function. The analysis of molecular data from SLE-Tregs identified a list of differentially expressed genes/molecular pathways that describes both the signature of dysfunctional Tregs and the ‘repairing effect’ of rapamycin. Altogether our data indicate that the functional defect of Tregs in SLE can be corrected along with their expansion in a therapeutic cell-product. Our findings suggest a new approach to use autologous Tregs in a programme of adoptive-cell-therapy for the treatment of SLE.

P.C4.02.14 Immune inhibitory CD200-Receptor potentiates type I immunity during inflammation
M. Van der Vlist1, M. Ramor2, L. van den Hooger3, S. Hidding4, M. van der Kruis5, R. Lebbink6, T. Radstake7, L. Meyaard8
1University Medical Centre, Utrecht, Netherlands, 2UMC Utrecht, Utrecht, Netherlands.

Immune responses are tightly regulated to allow pathogen clearance but prevent autoimmunity. Systemic Lupus Erythematosus (SLE) is an autoimmune disease that predominately affects women (9:1) and arises from dysregulated Toli-like receptor (TLR) signaling and aberrant T cell interferon production. The CD200 Receptor 1 (CD200R) is an inhibitory immune receptor that limits TLR-induced type I interferon responses especially in females. Therefore, we hypothesized that CD200R-mediated inhibition is absent in SLE patients. Surprisingly, we found that we observed in PBMC from SLE patients TLR-induced cytokine production was not suppressed by CD200R but was instead potentiated by it. CD200 signaling is impaired in TLR-induced cytokine production in healthy control (HC) PBMCs treated with IFNα. We found that in the absence of inflammation, CD200R inhibited Akt activation through Dock2 and Erk/pS6 activation through RasGAP, resulting in suppression of cytokine production. In contrast, SLE PBMC or HC PBMC treated with IFNα had decreased RasGAP to levels that were insufficient for CD200R to inhibit Erk/pS6. Furthermore, CD200R retained its ability to inhibit Akt via Dock2, which normally provides negative feedback on type I cytokine production. Our results indicate that SLE PBMCs are unable to downregulate IFNα-induced cytokine production through RasGAP, while leaving intact its ability inhibitory Akt. Taken together, these findings identify the signal transduction machinery of the immune-inhibitory receptor CD200R is responsive to type I IFNs thereby allowing CD200R to switch from inhibitory to potentiating depending on the inflammatory environment.

P.C4.02.15 Immunomodulatory properties of cellulose nanocrystals depend on their functionalization
M. Vasilev1, M. Bekic1, J. Ishimovski1, D. Mihalovic1, M. Milmanovic1, I. Majstorovic1, D. Ducevic2, S. Tomic3, M. Colic4,5,6
1Medical Faculty Focsa, University of Sarajevo, Focsa, Bosnia and Herzegovina, 2Institute for the Application of Nuclear Energy, Belgrade, Serbia, 3Medical Faculty of the Military Medical Academy, University of Defence, Belgrade, Serbia.

Cellulose nanocrystals (CNC) are attractive nanomaterials with large surface area suitable for development of drug delivery and diagnostic systems. However, the biocompatibility and immunomodulatory properties of CNC have not been studied so far, especially in relation to CNC functionalization. Here we used wood-based native (n)CNC, as precursors for TEMPO-oxidized (o)CNC and phosphorylated (p)CNC, to assess their toxicity and immunomodulatory potential on human peripheral blood mononuclear cells (PBMC) and monocyte-derived dendritic cells (MoDC). We found that non-toxic concentrations of 400 μg/ml of nCNC and oCNC impaired the proliferation and IL-2 production by phytohaemagglutinin-stimulated PBMC, whereas pCNC had no significant effects. According to CD14/CD1a expression analysis, oCNC displayed the strongest inhibitory effect on MoDC differentiation, followed by nCNC and pCNC, respectively. These results correlated with the weakest maturation capacity of oCNC-treated MoDC induced by LPS/IFNγ. Additionally, nCNC- and oCNC-treated MoDC expressed higher levels of CD14, CD83 and IL-12 compared to control MoDC, whereas pCNC-treated MoDC showed no such property. The capacity of MoDC to produce high levels of IL-12p70, IL-1b, IL-23, and low levels of IL-10, were impaired by nCNC and oCNC, but not by pCNC. In line with this, nCNC- and oCNC-treated MoDC displayed a decreased capacity to induce alloreactive T cells and TGF-β-producing DC4+CD25+FoxP3+ Treg cells, and a decreased capacity to induce IFNγ-producing Th1 cells in co-culture. Cumulatively, these results suggest that CNC may induce tolerogenic properties in MoDC, whereas phosphorylation of CNC prevents such effects, thus restoring the immunogenic potential of MoDC.

P.C4.02.16 Investigation of EZH2 as an epigenetic modifier of FoxP3 expression in regulatory T-cells in the light of immune tolerance impairment
A. Velichkov1, R. Susurkova1, A. Mihova1, M. Rakocevic2,3, M. Colic4,5,6
1Medical School and Methods: PBMCs from healthy controls (HCs) with no RPL: women with successful pregnancy (n=24; 23-45 years) and without pregnancy (n=10; 26-38 years) and patients with RPL (n=18; 25-44 years) were stained with anti-CD3/CD4/CD45RA/CD25/FOXP3 antibodies. FoxP3 and EZH2 expression were determined by PrimeFlowTM analysis.
2Materials and Methods: PBMCs from healthy controls (HCs) with no RPL: women with successful pregnancy (n=24; 23-45 years) and without pregnancy (n=10; 26-38 years) and patients with RPL (n=18; 25-44 years) were stained with anti-CD3/CD4/CD45RA/CD25/FOXP3 antibodies. FoxP3 and EZH2 expression were determined by PrimeFlowTM analysis.
3Institute for the Application of Nuclear Energy, Belgrade, Serbia, 4Laboratory of Molecular Genetics, Institute of Molecular Biology "R. Tsanev", 5Bulgarian Academy of Sciences, Sofia, Bulgaria.

Introduction: Regulatory T-cells (Tregs) represent the effective arm of immune tolerance. Their function is tightly regulated and its impairment is associated with recurrent pregnancy loss (RPL), autoimmune and allergic reactions. Our aim is to analyze EZH2, an epigenetic modifier of FoxP3 expression by the model of recurrent pregnancy loss. Materials and Methods: PBMCs from healthy controls (HCs) with no RPL: women with successful pregnancy (n=24; 23-45 years) and without pregnancy (n=10; 26-38 years) and patients with RPL (n=18; 25-44 years) were stained with anti-CD3/CD4/CD45RA/CD25/FOX3P antibodies. FoxP3 and EZH2 expression were determined by PrimeFlowTM assay and qRT-PCR in non-stimulated or stimulated with progressively increased progesterone concentrations. The FACs analysis was done using FlowJo V10 and the Statistical analysis by GraphPad Prism7.

Results: The overall analysis showed lower CD45RA+FOX3P+Tregs percentages in patients compared to the healthy groups (p<0.05). The highest proportion of CD25+Tregs was found in the group of women w/o pregnancy (p<0.05). Conversely, the proportion of CD45RA+FoxP3-CD25-TCs dominated in the group of patients (p<0.05). The percentage of EZH2+FoxP3- cells in non-Tregs and Tregs was greater in HCp (p<0.05) and showed progesterone-dependent variations.
Conclusion: Our results suggest that the pregnancy impacts mainly nCD4+ population. Epigenetic modifications facilitated by the hormonal milieu might be associated with variations in FoxP3 expression. Considering that EZH2 and FOXP3 are indispensable for the fate of Treg, further investigations are in line to clarify the precise regulation mechanisms in Tregs. Acknowledgements: This work was supported by Grant DN03/4-2016 of National Science Fund, Ministry of Education and Science, Republic of Bulgaria.

P.C4.02.17 Genome-wide methylation analysis of regulatory and conventional T cells
A. Salumets1, H. Peterson1, P. Peterson1
1Institute of Computer Science, Tartu, Estonia, 2Institute of Biomedical and Translational Medicine, Tartu, Estonia.

Regulatory T cells (Tregs) represent a subpopulation of T cells that are specialised in immune suppression and maintenance of immune tolerance. They have a crucial role in the prevention of autoimmunity and their failure leads to development of autoimmune diseases. We compared the Tregs to their conventional counterparts (Tconv) that serve an opposing role - activation of immune system. The aim of our study was threefold: [1] to examine differentially methylated positions (DMPs) and regions (DMRs) between Tregs and Tconv; [2] find DMPs and DMRs between Tregs from healthy controls and Graves’ patients; and [3] find which DMPs and DMRs from the previous step are characteristic to Tregs. To meet the objectives, we performed a genome-wide methylation analysis with Infinium Human Methylation EPIC BeadChips. Firstly, we focused on CpGs that were differentially methylated between Tregs and Tconv (6 healthy controls). As a result, we found nearly 19,000 DMPs and 630 DMRs (FDR<0.05). Secondly, we investigated differences between Tregs from healthy controls (10 individuals) and Graves’ patients (11 individuals) which resulted in 19 DMRs (FDR<0.05). Our analysis indicated no overlap between those two sets of DMPs. However, we observed differences between methylation value distribution of Tconv and Tregs where Tconv had more extreme methylation values i.e. they had more CpGs with either low or high methylation level.

Acknowledgements: This work was supported by Grant DN03/4-2016 of National Science Fund, Ministry of Education and Science, Republic of Bulgaria.
Inflammation is often associated to a hypoxic state imposing a metabolic constraint on inflammatory cells. The protein HIF1α plays an important role in cells hypoxia adaptation, and it is upregulated by the oxygen-sensing prolyl-hydroxylase 2 (PHD2) in numerous cell types including T lymphocytes. The impact of hypoxia on immune cells, in particular on regulatory T cell (Treg) function, has not been fully elucidated. The purpose of our study is to evaluate the role of the PHD2-HIF1α axis in the regulation of homeostasis and function of Tregs.

We demonstrate in this work that selective ablation of PHD2 expression in Tregs (PHD2ΔCD4+) mice leads to a spontaneous intestinal inflammatory syndrome, as evidenced by the development of a rectal prolapse and elevated expression of Il-1β and Il-10 in the mesenteric lymph nodes and spleen. PHD2 deficiency in Tregs leads to an increased number of activated CD4 and CD8 conventional T cells expressing an effector-like phenotype (CD44^CD62L^-). Concomitantly, the expression of innate-type cytokines such as Il-1β, Il-12p40, Il-12p35 and TNF-α is found to be elevated in peripheral (p)Tregs. Finally, PHD2ΔCD4+ mice display an enhanced sensitivity to DSS-induced colitis and to experimental autoimmune encephalomyelitis (EAE), suggesting that PHD2-deficient Tregs do not efficiently control inflammatory response in vivo. The mechanisms whereby PHD2 controls Treg activity is presently under investigation, in particular through the development of in vitro models of immune suppression. We hope that our study will contribute to a better understanding of the role of oxygen-sensing pathways in the regulation of inflammatory responses.

P.C4.03.02 Short term cold acclimation enhances human Treg induction

M. Becker1,2, I. Serr1,2, L. Mengel1, H. Hauner1,2, M. H. Tschöp1,2, C. Daniel1,2;
1Institute of Diabetes Research, Group Immune Tolerance in Diabetes, Helmholtz Diabetes Center at Helmholtz Zentrum München, German Research Center for Environmental Health, Munich, Germany; 2German Center for Diabetes Research (DZD), Munich, Germany; 3EIEL-Institute for Food & Health, Else Krämer-Fresenius Zentrum für Ernährungsmedizin, Technische Universität München, Freising-Weihenstephan, Germany; 4German Institute of Human Nutrition Potsdam-Rehbrücke, Nutrition and Clinical Research, Potsdam, Germany.

Inhibitory Anti-Drug Antibody (ADA) responses interfere with Factor VIII replacement efficacy in 25-30% of Hemophilia A (HA) cases, greatly increasing patient morbidity and costs. We investigated the role of ADA responses in patients with or without severe GH (anti-FVId) antibodies. We observed an increase in Treg frequency and memory Treg CD45RA(-)FoxP3(hi) frequency in patients with a history of cancer, compared to patients without cancer. We analyzed by flow cytometry blood samples from 60 renal transplant recipients for more than 10 years, without signs of rejection, treated with minimized immunosuppressive treatments, and explored their profile associated with adenocarcinomas. We hope that our study will contribute to a better understanding of the mechanisms underlying operational tolerance in transplantation.

P.C4.03.03 Salt modulates cellular metabolism of regulatory T cells

B. F. Côté-Rea1,2, O. Matveeva-Kolm1, I. Hamad1, A. Gurevits13, L. Dubais1, M. Kleinefeidfeld1; 1VIB Laboratory for Translational Immunomodulation, VIB Center for Immunology Research (VIRI), Hasselt, Belgium; 2Department of Radiation Oncology (Maastro), GROW - School for Oncology and Developmental Biology, Maastricht, Netherlands.

Salt modulates cellular metabolism of regulatory T cells in vitro. Moreover, human CD4+CD25+FOXP3+ T cells in vitro. Moreover, human CD4+CD25+FOXP3+ T cells express key components of the molecular interface connecting adipose tissue (AT) function with environmental cold or low-dose beta3-adrenergic stimulation. Specifically, by loss- and gain-of-function experiments, including Treg depletion and transfers in vivo, we identified a T-cell-specific Stat6/PTEN signaling axis that links cold exposure or beta3-adrenergic stimuli with tissue AT function and AT function, respectively. However, the translational relevance of these findings for human Treg induction in response to beta3-adrenergic stimulation or cold remains currently unknown. Here, we show that beta3-adrenergic stimulation using Mirabegron (Mira) induces human Tregs in a preclinical setting only if the donor mice express human CD3CD4CD122CD25foxp3+Tregs (of CD4+T cells); control: 1.9±0.5 vs. Mira: 4.9±1.0; p=0.0319) accompanied by increased Treg induction potential from naive CD4+T cells in vitro. Moreover, human CD4+T cell analyses of subcutaneous AT biopsies after an acute cold stimulus of 2 hours to healthy subjects provide first evidence for an increase in local CD3CD4CD122CD25foxp3+Tregs (Tregs [of CD3CD4]): t0=1.5±0.04% vs. t2=3.1±0.08%). Of note, short-term human cold acclimation in vivo also enhanced human Treg induction potential from naive CD4+T cells in peripheral blood (CD3CD4CD122CD25foxp3)Tregs [of CD4]: t0=0.100±0.7% vs. t2=137±125%; p=0.030). These findings support the concept that cold exposure or beta3-adrenergic stimulation can exert pro-tolerogenic functions on human CD4+T cells. Further mechanistic analyses are required to dissect molecular underpinnings of human Foxp3+Treg induction in response to cold or beta3-adrenergic stimulation in health and metabolic disease.
P.C.4.03.06
Functional defect of regulatory T cells in Anti-Neutrophil Cytoplasmic Autoantibody associated vasculitis is associated with overexpression of microRNA-142-3p
G. Dekkema1, T. Bjinj2, W. H. Abdullaho3, P. G. Jellema1, A. Van Den Berg3, B. Kroesen4, C. A. Stegeman5, P. Heeringa6, J. Sanders7,
1Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, Netherlands, 2Department of Internal Medicine, division of Nephrology, University Medical Center Groningen, Groningen, Netherlands, 3Department of Rheumatology and Clinical Immunology, University Medical Center Groningen, Groningen, Netherlands, 4Department of Clinical Immunology, University Medical Center Groningen, Groningen, Netherlands.

Introduction: Circulating regulatory T cells (Tregs) in anti-neutrophil cytoplasmic antibody associated vasculitis are frequently functionally deficient. The mechanism behind their impaired function is however unknown. Here, we hypothesized that the dysfunctionality of Tregs in AAV is due to altered microRNA (miR) expression in these cells.

Methods/Results: Tregs (CD4+CD45ROCD25+CD127-) of healthy controls (HC) and AAV patients in remission without treatment (AAV-REM) were FACS-sorted, and total RNA was isolated. Samples from 8 HC and 8 AAV-REM subjects were subjected to miRNA microarray analysis. Nineteen miRNAs were differentially expressed, and in an independent validation cohort, miR-142-3p was confirmed to be significantly upregulated (2-fold, p<0.03) in Tregs of AAV-REM patients (n=23). In vitro transient overexpression of miR-142-3p using a mimic-miR-142-3p showed that the suppressive capacity of Tregs was significantly enhanced upon overexpression (1.9-fold reduction, p<0.01), and miR-142-3p levels tended to negatively correlate to the suppressive power of Tregs (p=0.06, rho=0.591). A database and literature search identified adenylyl cyclase-9 (AC9) as validated target for miR-142-3p. miRNA levels of AC9 (3.8-fold) tended to be lower in AAV-REM Tregs. In addition, cyclic AMP (cAMP) levels in Tregs, partly produced by AC9, were measured after acCD3/CD28 stimulation. After 48 stimulation, cAMP levels were significantly lower in AAV-REM Tregs (1.7 fold, p<0.003). Moreover, overexpression of miR-142-3p also significantly lowered the cAMP production.

Conclusion: Increased expression of miR-142-3p in Tregs of AAV-REM patients may induce their functional deterioration by targeting the AC9/cAMP mediated suppression.

P.C.4.03.07
In vivo screening of novel fusion proteins targeting CD28 and PD1 pathways inhibiting immune responses and promoting long-term transgene expression in the context of muscle gene therapy
L. Dupaty1, M. Demelles2, G. Iou3, L. Jean1, A. Savettieri4, O. Buyer1, S. Adriaouch5,
1Normandie Univ, UNIROUEN, INSERM, U1234, Physio-pathologie, Auto-immunité, maladies Neuromusculaires et THérapiés Régénératrices (PANTHER), Rouen, France, 2Cancer Research Center of Lyon (CRCL), INSERM U1252, CNRS UMR5206, Lyon, France.

In vivo gene therapy meditated by adeno-associated viral (AAV) vectors becomes a feasible therapeutic strategy in human. However, immune responses against the therapeutic gene products (tgP) represent a major concern as this method widened to a spectrum of pathologies and individuals. One remaining challenge is to induce immunological tolerance towards the tgP.

For that, we implemented a stringent animal model and evaluated a panel of 11 novel fusion proteins derived from CTLA-4 and/or PD1P with the aim to manipulate these major immunoregulatory axes and to induce long-term tolerance. To directly screen in vivo the immunoregulatory properties of our selected protein candidates, we used AAV vectors to produce them in vivo and co-injected them together with an AAV vector coding for the strongly immunogenic Ovalbumin tgP. This screening strategy allowed the identification of 2 immunoregulatory candidates, PD1-11 and PD1-12, that significantly inhibit cellular and antibody responses against the tgP and, remarkably, improved Ova persistence in vivo. Interestingly, and in contrast to CTLA-4/Fc, these proteins preserve the Treg compartment and are associated with active immunoregulation rather than inhibition of lymphocytes priming.

Finally, based on our results, we implemented sequential strategy relying on a single injection of CTLA-4/Fc, to inhibit the priming of initial immune response, subsequently followed by the injections of our selected PD1-11 or PD1-12 immunoregulatory proteins. This strategy may be of interest in gene therapy as well as in transplantation where long-term tolerization remains a major challenge.

P.C.4.03.08
A novel approach for the isolation of medullary thymic epithelial cells from murine thymi improves purity and cell recovery
R. Engelmann6, D. Dohr1, B. Müller-Hilke7,
6Institute of Immunology & Core Facility for Cell Sorting and Cell Analysis, Rostock, Germany, 7Institute of Immunology, Rostock, Germany.

Objective: Medullary thymic epithelial cells (mTEC) play a central role in the removal of T cells specific for tissue-restricted antigens and thus prevent disastrous autoimmunity. Thus methods to efficiently isolate these cells are warranted.

Methods: Excised murine thymi were digested with a standard dispase/collagenase/DNase mixture. Thereafter, mTEC were magnetically enriched using UEA-1 microbeads and CD45+ thymocytes were subsequently depleted by cell straining. This novel method was compared to the broadly used enrichment by percoll density gradient centrifugation followed by flow cytometry cell sorting. Results: The usage of 2µl UEA-1 beads per 1x10^6 cells for magnetic enrichment was superior to the percoll method in terms of the percentage of enriched mTEC (22% versus 1.2% on average) and the number of isolated mTEC per thymi (2x10^5 versus 5x10^4). The viability after both procedures was comparable. Subsequent depletion of CD45+ cells resulted in an mTEC purity of 74% compared to 97% after flow cytometric cell sorting. However, the recovery rate of cells proved to be significantly higher after cell straining as compared to flow cytometric cell sorting. Conclusions: The combination of magnetic enrichment of UEA-1+ cells with subsequent depletion of CD45+ cells via cell straining resulted in increased yields of mTEC as compared to the current gold standard method.

P.C.4.03.09
Prostaglandin E2 potentiates the suppressive functions of human GM-CSF/IL-6-induced myeloid-derived suppressor cells in vitro
B. Joksimovic1, M. Colic2,1,3, M. Volkov4, L. Hafkenscheid5, A. Kempers6, M. A. van Delft7, T. M. Huizinga8, D. van der Woude9, R. E. Toes10, G. Dekkema11, P. C.4.03.09,
1Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, Netherlands, 2Department of Rheumatology and Clinical Immunology, University Medical Center Groningen, Groningen, Netherlands, 3Department of Clinical Immunology, University Medical Center Groningen, Groningen, Netherlands.

MDSC Myeloid derived suppressor cells (MDSC), discovered as one of the major factors driving tumor progression, are also involved in beneficial effects during the course of autoimmune Medical Academy, University of Defence, Belgrade, Serbia.

Introduction: The immense role of prostaglandin (PG)-E2 in the induction of MDSC was confirmed as one of the major factors driving tumor progression. MDSC, however, are also involved in beneficial effects during the course of autoimmune Medical Academy, University of Defence, Belgrade, Serbia.

Methods: Results: Tregs (CD4+CD45ROCD25+CD127-) of healthy controls (HC) and AAV patients in remission without treatment (AAV-REM) were FACS-sorted, and total RNA was isolated. Samples from 8 HC and 8 AAV-REM subjects were subjected to miRNA microarray analysis. Nineteen miRNAs were differentially expressed, and in an independent validation cohort, miR-142-3p was confirmed to be significantly upregulated (2-fold, p<0.03) in Tregs of AAV-REM patients (n=23). In vitro transient overexpression of miR-142-3p using a mimic-miR-142-3p showed that the suppressive capacity of Tregs was significantly enhanced upon overexpression (1.9-fold reduction, p<0.01), and miR-142-3p levels tended to negatively correlate to the suppressive power of Tregs (p=0.06, rho=0.591). A database and literature search identified adenylyl cyclase-9 (AC9) as validated target for miR-142-3p. miRNA levels of AC9 (3.8-fold) tended to be lower in AAV-REM Tregs. In addition, cyclic AMP (cAMP) levels in Tregs, partly produced by AC9, were measured after acCD3/CD28 stimulation. After 48 stimulation, cAMP levels were significantly lower in AAV-REM Tregs (1.7 fold, p<0.003). Moreover, overexpression of miR-142-3p also significantly lowered the cAMP production.

Conclusion: Increased expression of miR-142-3p in Tregs of AAV-REM patients may induce their functional deterioration by targeting the AC9/cAMP mediated suppression.

P.C.4.03.10
Post-translational modified protein antibodies in Rheumatoid arthritis: searching for the eye of the storm

Autoantibodies against post-translationally modified proteins (Anti-MAP antibodies or AMAP) are a hallmark of Rheumatoid Arthritis (RA). A variety of AMAPs against different protein modifications, such as citrullinated proteins, carbamylation proteins and acetylated proteins have now been described in RA. Since these antibodies are far less frequently found in healthy persons with other autoimmune diseases, a shared "developmental" basis is suggested. At present, the origin or mutual relationship of AMAPs is poorly understood. Here, we aimed to study the origin of AMAP-responses by postulating that the AMAP-response shares a common "background" that evolves over time into different classes of AMAPs. Immunisation of mice with carbamylated proteins not only induced an antibody response recognizing carbamylationated proteins, but also acetylated proteins. Similarly, also immunization with acetylated proteins led to the formation of (auto)reactive AMAPs against other modifications as well. Analysis of antibodies purified with citrullinated antigens (ACPs) from blood of RA patients/blood of healthy persons revealed that ACPs, besides citrulline-reactivity, can also display reactivity to acetylated and carbamylationated peptides. Similarly, purified anti-citrulline protein antibodies showed cross-reactivity against all three post-translational modifications. Our data show that different AMAP-responses can evolve from exposure to only one type of modified protein. These findings indicate that the different AMAP-responses originate from a common "precursor" B cell response that diversifies into multiple distinct AMAP-responses over time and explain the presence of multiple AMAPs in RA, one of the hallmarks of disease.
Regulatory T cells (Treg) exert contact-dependent inhibition of immune cells through the production of active TGF-β1. This immunosuppressive cytokine is secreted by all immune cells as a latent and inactive form, in which the mature cytokine is associated to the Latency Associated Peptide (LAP), that precludes the interaction of the mature cytokine with its receptor. To exercise its activity, mature TGF-β1 must be released from the LAP, a process referred to as TGF-β1 activation. Tregs activate latent TGF-β1 via a mechanism that requires GARP, a transmembrane protein called GARP, which binds latent TGF-β1 by forming disulfide bonds with LAP. We wish to derive monoclonal antibodies that activate latent TGF-β1 by binding to GARP/latent TGF-β1 complexes, to provide means to stimulate Treg functions in vivo. If we succeed, we will attempt to obtain the crystallographic structure of GARP/latent TGF-β1 in complex with our activating antibody. We intend to identify the tri-dimensional changes in GARP/latent TGF-β1 complexes that lead to the release of active TGF-β1 and obtain information on the mode of action of the antibody. We tested 81 monoclonal antibodies directed against GARP, latent TGF-β1 or GARP/latent TGF-β1 complex in vitro. Only 7 were capable of activating TGF-β1 from GARP/latent TGF-β1 complexes. All these antibodies bind murine latent TGF-β1 regardless the presence of Garp. After analysis of binding and activating properties of these anti-mouse latent TGF-β1 antibodies, we started to confirm their activity in vitro on murine cell populations expressing Garp/latent TGF-β1 complexes such as CD4+ T lymphocytes or platelets.

Tolerogenic dendritic cell (tolDC) therapy is a promising strategy for the attenuation of pathogenic T cells in autoimmune diseases such as multiple sclerosis (MS). Our group has developed an autologous antigen-specific cell therapy based on vitamin D3 (ViTD3)-tolDC loaded with myelin peptides. OBJECTIVE: To describe the design of a multicenter, open-label, dose-escalation Phase I clinical trial to evaluate feasibility, safety, tolerability and preliminary efficacy of intranodal administration of ViTD3-tolDC in active MS patients. METHODS: In vitro studies have demonstrated a dose-dependent regulatory activity of ViTD3-tolDC reducing lymphocyte proliferation and IFN-γ production and increasing IL-10 levels, in co-culture experiments. Moreover, in vivo studies in the animal model of MS revealed a beneficial effect of ViTD3-tolDC ameliorating the severity of the disease. Considering these pre-clinical results, a clinical trial was designed. RESULTS: Active MS patients will be included in a dose-escalation best-of-five design: Cohort 1 (5x10^6 ViTD3-tolDC), Cohort 2 (10x10^6), Cohort 3 (15x10^6). A fourth Cohort of patients under IFN-beta treatment receiving the selected dose of ViTD3-tolDC will be included. The trial protocol has been approved by the Spanish regulatory authorities (AEMPS) (https://clinicaltrials.gov/ct2/show/NCT02903537). Each cohort will receive 6 administrations of tolDC (first 4 every 2 weeks and last 2 every 4 weeks). Clinical, MRI and immunological monitoring of 12 patients will be performed for 24 months. Each patient will be its own pre- and post- intervention control.

CONCLUSIONS: Positive outcomes of this phase I clinical trial may lead to a phase II trial to investigate the efficacy of this therapy in MS patients.
Ankle circumference, clinical and histological scores decreased in CIA mice which are treated with pELAM-1pro/hIDO. The ratios of CD4+ plasmid and controls with liposomes intraarticularly delivered after arthritis developed in mice and followed up for 4 weeks. The therapeutic effects evaluated by considering HeLa cells were transfected with pELAM-1pro/hIDO and induced with IL-1β for 6 or 24 hours. hIDO levels were determined from the cell lysate. The combination of therapeutic treatment responses of disease-inducible IDO gene over expression in CIA mice were investigated. Human IDO cDNA mammalian expression plasmid (pCMV/hIDO) CMV opening new avenues to explore its therapeutic use for preventing NTN in humans.

CD4+ FoXP3+ regulatory T cell-mediated immunomodulation by pharmacological inhibition of the acid sphingomyelinase in humans

W. Winter, F. Deenstäd, C. Holsmann, S. Stonewski, C. Wurts, M. Buttmann, A. Menkel, J. Schneider-Schaulies, N. Beyer-Dorsch. 1University of Würzburg, Institute for Virusology and Immunobiology, Vorschartenstr. 7, Würzburg, Germany, 2University Hospital Würzburg, Department of Psychiatry, Psychosomatics and Psychotherapy, Margarete-Höppel-Platz 1, Würzburg, Germany, 3Caritas Hospital Bad Mergentheim, Department of Neurology, Uhlandstraße 7, Würzburg, Germany, 4University Hospital Würzburg, Department of Neurology, Josef-Schneider-Str. 11, Würzburg, Germany.

The acid sphingomyelinase (ASM) is a key modulator of cellular signaling pathways in which bioactive sphingolipids play crucial roles by catalyzing the cleavage of sphingomyelin to ceramide and phosphocholine. Recently, we have demonstrated that genetic deficiency for or pharmacological inhibition of the ASM increases the activity and frequency of mouse FoXP3+ regulatory T cells (Treg) among CD4+ T cells. Furthermore, pharmacological inhibition of the ASM had beneficial effects in different mouse models of autoimmune and inflammatory diseases. In the present study, we performed in vitro experiments with human T cells using two widely prescribed antidepressants with high (sertraline) or low (citalopram) capacity to inhibit ASM activity. Similar to our findings in mice, ASM inhibition in human PBMC increased the frequency of Treg among human CD4+ T cells. To assess whether these effects on human T cells are transferable in vivo, we have been prospectively analyzing the composition of CD4+ T cells in patients treated for major depression. Our preliminary data show that pharmacological inhibition of the ASM is superior to anti-depressants with little or no ASM-inhibitory activity in normalizing effector Treg frequencies among CD4+ T cells in patients treated for depression. In summary, we find that inhibition of the ASM increases the frequency of (effector) Treg among CD4+ T cells in mice and humans suggesting that ASM blockade might beneficially modulate autoimmune diseases and depression-promoting inflammatory reactions. This study was supported by a grant from the DFG (FOR2123 project PO1).

The effect of IL-2/anti-IL-2 complex treatment on antigen presenting cells

M. Willet, B. Mohr, N. Granofsky, M. Muckenhuber, T. Wekerle, J. Sprent, N. Pilot. 1Department of Surgery, Medical University of Vienna, Vienna, Austria, 2Institute of Medical Research, Sydney, Australia, 3University of Sydney, Sydney, Australia.

Introduction: The use of interleukin-2 (IL-2) complexed with a specific antibody against IL-2 (IL-2cplx) has been shown to selectively increase regulatory T cells (Tregs) without significantly proliferating other IL-2 responsive immune cells. Here we focused on the effect of IL-2cplx treatment on antigen presenting cells (APCs) and changes in the expression of molecules relevant for immune response activation.

Methods: C57BL/6 mice received IL-2cplx (1µg/5µg), i.p., for 3 consecutive days and were sacrificed on day 5. We used flow-cytometric analysis to investigate the frequency of Treg and APCs focusing on the expression of relevant markers on APCs for immune response activation.

Results: We demonstrate that IL-2cplx led to significant expansion of Tregs (4.6% vs 0.04% p<0.0008; ns naive) and changes in the frequency of DC1+ APCs (20.1% vs 0.94% naive) in our model. Moreover we demonstrated significantly lower expression of CD86 and CD40 (16.4% vs 22.8% p=0.02 and 29.8% vs 34% p=0.05; ns naive) and significantly higher expression of MHC class II on DC1+ in CD86 (82.9% vs 75.35% p=0.009; ns naive).

Conclusion: Treatment with IL-2cplx and subsequent expansion of Tregs leads to reduced expression of CD80+ and CD86+ but increased MHC class II expression on APCs which could cause impaired effector T cell function. This may highlight possible ligand-receptor interactions and help to understand important cellular key mechanisms mediated by APCs and T cells.

Treatment of collagen-induced arthritis (CIA) in mice model with disease-inducible indoleamine-2,3-dioxygenase (IDO) gene

I. Yilmaz, G. Guven, M. Karayaz, A. O. Baraz, F. Erayci, C. Akko, A. Akko, A. Yilmaztepe Oral, M. Yalçın, H. B. Oral. 1Department of Medical Immunology, Institute of Health Sciences, Uludag University, Bursa, Turkey, 2Department of Veterinary Physiology, Institute of Health Sciences, Uludag University, Bursa, Turkey, 3Department of Immunology, Institute of Health Sciences, Gazi University, Ankara, Turkey, 4Department of Veterinary Physiology, Institute of Health Sciences, Uludag University, Bursa, Turkey, 5Department of Veterinary Physiology, Institute of Health Sciences, Uludag University, Bursa, Turkey, 6Department of Veterinary Physiology, Faculty of Medicine, Uludag University, Bursa, Turkey, 7Department of Veterinary Physiology, Faculty of Medicine, Uludag University, Bursa, Turkey.

In RA patients, tryptophan catabolism has an important role on progression and interleukine-2,3-dioxygenate (IDO) has a crucial role in the induction of immune tolerance. In this study, the treatment responses of disease-inducible IDO gene over expression in CIA mice were investigated. Human IDO cDNA mammalian expression plasmid (pCMV/hIDO) CMV promoter (constantly active) was replaced with pELAM-1 promoter (only active in the presence of inflammatory cytokines) which was taken out from pELAM-1pro/AT plasmid. HeLa cells were transfected with pELAM-1pro/Hido and induced with IL-1β for 6 or 24 hours. HDI levels were determined from the cell lysate. The combination of therapeutic plasmid and controls with liposomes intraarticularly delivered after arthritis developed in mice and followed up for 4 weeks. The therapeutic effects evaluated by considering ankle circumference, clinical and histopathological scoring of mice. Moreover, the difference between CD4+ T cell and CD68+ synovial macrophages amounts, and IDO expression in joints was examined. It was observed that HDI was significantly increased following 6 and 24 hours stimulation with IL-1β in HeLa cells transfected with pELAM-1pro/Hido. Ankle circumference, clinical and histological scores decreased in CIA mice which are treated with pELAM-1pro/Hido. The ratios of CD4+ T cells and CD68+ synovial macrophages decreased and IDO levels increased following pELAM-1pro/Hido treatment. Thus, conditional targeting IDO can be a new approach for the treatment of RA. This study is supported by The Scientific and Technical Research Council of Turkey (TUBITAK-COST Project No: 113S375) under Cost Action BM1305. COST is supported by the EU Framework Programme Horizon 2020.
P.C5.01 Allergy, asthma and therapy - Part 1

P.C5.01.01

Loading of the lipocalin BLG with iron-quirin complex prevents the onset of allergy in BALB/c mouse model

S. M. Affii1, I. Pol-Schöll1, G. Hofstetter1, A. Vidovic1, L. Pacias1, F. Roth-Walter1, E. Jensen-Jarolim1

1The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria and Laboratory Medicine and Immunology Department, Faculty of Medicine, Menoufia University, Menoufia, Egypt, 2The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria, 3Biotecnology-Vegetal Biology Department, ETSIaAB and Center for Plant Biotechnology and Genomics (CBGP; UPN-INA), Technical University of Madrid, Madrid, Spain, 4The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Vienna, Austria.

Background: Prevention of milk allergy is an urgent problem that has attracted the attention of food scientists. In previous studies we proved that the unloaded apo-form of the lipocalin beta-lactoglobulin (BLG) from milk promoted TH2 cells and inflammation, whereas the holo-form acted immunosuppressive. In this study, we tested in BALB/c mice whether nasal application of holo-BLG can prevent allergy to BLG.

Methods: BALB/c mice were sensitized twice intraperitoneally with BLG adjuvanted with aluminum hydroxide after being nasally treated 3 times in biweekly intervals with the unloaded apo-form of BLG, or holo-BLG loaded with quercetin-iron complexes, or water as sham-treatment. Then mice were intraperitoneally challenged with apo-BLG.

Results: A single high-dose feeding with holo-BLG prevented allergic sensitization to BLG. Mice pretreated with water or apo-BLG had significantly elevated BLG-specific antibodies, whereas holo-BLG pretreated mice showed reduced levels of BLG-specific antibodies as evaluated by ELISA. MHC Class II+Ad and CD86+ expression on CD11c+ dendritic cells from spleens were analyzed by flow cytometry. Results: Intranasal prophylactic treatment with holo-BLG prevented allergenic sensitization to BLG. Mice pretreated with water or apo-BLG had significantly elevated BLG-specific antibodies, whereas holo-BLG pretreated mice showed reduced levels of BLG-specific antibodies as evaluated by ELISA. MHC Class II+Ad and CD86+ expression on CD11c+ dendritic cells from spleens were analyzed by flow cytometry.

Conclusion: Prophylactic treatment with holo-BLG provided specific protection against sensitization to BLG and prevented the onset of allergy.

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P.C5.01.02

Analysis of IF4-binding characteristics using a streptavidin-biotin-dependent “bio-ChIP”

S. Dietzen, T. Bopp

University Medical Center of the Johannes Gutenberg-University, Institute for Immunology, Mainz, Germany.

Introduction: Basophils are required for IgE-mediated chronic allergic skin inflammation (IgE-CAI) in the mouse and promote the accumulation of eosinophils in tissues. However it is not clear how eosinophils are recruited and if hemelnh-induced IgE is protective in this setting.

Material and Methods: Here, we used an antibody-induced model of IgE-CAI in mice. 3µg IgE antibody against TNP was injected intravenously followed by intradermal injection of 10µg TNP-OVA in the ear the next day. We measured changes in ear thickness and recruitment of effector cells into the ear parenchyma and compared hemelnh infected vs. naive and several transgenic mouse strains.

Results: The ear swelling was absent in basophil- and eosinophil-deficient mice as well as in mice lacking the high affinity receptor for IgE on basophils. Swelling decreased in mice infected with Nippostrongylus brasiliensis and Helastrongylosomoides polygyrus compared to wildtype mice. Further IL-4/IL-13-deficient mice, as well as Stat6-deficient mice showed slightly decreased ear swelling. Infiltration of basophils, eosinophils, and neutrophils was also impaired.

Conclusion: From our studies we can conclude that in IgE-CAI basophils are activated via the IgE receptor and promote the recruitment of the recruited IgE-CAI basophils from hemelnh-induced IgE appears to protect from IgE-CAI.

P.C5.01.03

Helminth protect from basophil- and eosinophil-mediated chronic allergic skin inflammation

J. U. Eberle, D. Voehringer;
Department of Infection Biology, University Hospital Erlangen and Friedrich-Alexander University Erlangen-Nuremberg (FAU), Erlangen, Germany.

Introduction: IgE-mediated inflammatory diseases such as allergy, asthma, and several transgenic mouse strains.

Results: The ear swelling was absent in basophil- and eosinophil-deficient mice as well as in mice lacking the high affinity receptor for IgE on basophils. Swelling decreased in mice infected with Nippostrongylus brasiliensis and Helastrongylosomoides polygyrus compared to wildtype mice. Further IL-4/IL-13-deficient mice, as well as Stat6-deficient mice showed slightly decreased ear swelling. Infiltration of basophils, eosinophils, and neutrophils was also impaired.

Conclusion: From our studies we can conclude that in IgE-CAI basophils are activated via the IgE receptor and promote the recruitment of the recruited IgE-CAI basophils from hemelnh-induced IgE appears to protect from IgE-CAI.

P.C5.01.04

A single high-dose feeding with the major carp allergen parvalbumin induces immunological and clinical tolerance in a mouse model of fish allergy

R. Friedl1, A. Gstoettner1, U. Baranyi2, J. Savodova1, G. Stavroulakis1, N. Papadopoulos1, F. Stal1, M. Focke-Teijl1, T. Wekerle1, R. van Reel2, R. Volenta1, B. Linhart1

1The Division of Immunopathology, Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, 2The Department of Transplantation Immunology, Department of Surgery, Medical University of Vienna, Vienna, Austria, 3Allergy Research Center, 2nd Pediatric Clinic, University of Athens, Athens, Greece, 4Division of Infection, Immunology & Respiratory Medicine, University of Manchester, Manchester, Manchester, United Kingdom, 5Biomay AG, Vienna, Austria, 6Department of Experimental Immunology and of Otorhinolaryngology, Academic Medical Center, Amsterdam, Netherlands.

Introduction: Intestinal feeding with the major carp allergen parvalbumin (nCyp c 1) on the antibody responses and allergic symptoms in a fish allergy mouse model. Materials and methods: BALB/c mice (n=8) were fed with 10mg carp parvalbumin (Cyp c 1) for 3 months of age. Thus a prophylactic strategy for prevention of IgE-sensitization to food allergens in infants is the ultimate goal. This study sought to investigate the effect of feeding of natural carp parvalbumin (nCyp c 1) on the antibody responses and allergic symptoms in a fish allergy mouse model.

Results: Following a single high-dose feeding with nCyp c 1 a prophylactic strategy for prevention of IgE-sensitization was achieved. Further, Cyp c 1-specific antibodies were not analyzed in ELISA and rat basophilic leukemia assay. Results: Measurement of Cyp c 1-specific antibodies demonstrated that IgE-sensitization was prevented by prophylactic feeding with nCyp c 1. Further, mice fed with nCyp c 1 before sensitizations were protected from symptom-development upon allergen challenge. Conclusions: A single high-dose feeding with carp parvalbumin induced tolerance in our mouse model. The induction of clinical tolerance to allergens was investigated in numerous oral immunotherapy trials. However, the application of natural allergens causes severe symptoms in patients and clinical tolerance is often not persistent. We suggest prophylactic tolerance induction as alternative strategy. Support: FAST-project 201871 and Austrian Science Fund-projects P23350-B11, F4605.

P.C5.01.05

Comprehensive tracking of mediator reprogramming in type 2 immune settings reveals macrophage eosinocaid plasticity during allergen exposure

A. Friedl1, F. Henkel1, D. Thomas2, T. Bouchery4, P. Haier1, C. B. Schmidt-Weber1, J. Adamski6, N. L. Harris1, M. Haifl; J. Isser-van Bieren1

1Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Munich, Germany, 2Pharmazentrum Frankfurt/ZAFES, Institute of Clinical Pharmacology, University Frankfurt, Frankfurt, Germany, 3Biomay AG, Vienna, Austria, 4Department of Infection Biology, University Hospital Erlangen and Friedrich-Alexander University Erlangen-Nuremberg (FAU), Erlangen, Germany, 5Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Center Munich, Neuherberg, Germany, 6Chair of Experimental Genetics, Life and Food Science Center Weihenstephan, Technical University of Munich, Freising-Weihenstephan, Germany.

Background: Eicosanoid lipid mediators play key roles in allergy and asthma. Macrophages represent major cellular sources of these mediators, but their complex and dynamic eicosanoid output during type 2 immune responses is poorly understood. Objective: We aimed to comprehensively characterize macrophage eicosanoid output using type 2 immune responses. Methods: We established an LC-MS/MS workflow for the quantification of 52 oxylipins to track lipid mediator reprogramming in human monocyte derived macrophages (MDM) during exposure to house dust mite (HDM) or in nematode infection in vivo. Eicosanoid enzymes were studied by qPCR and westernblot and cytokine production was assessed by multiplex assays. Results: Differentiation of macrophages with GM-CSF and TGFB1 resulted in a phenotype (“aMDM”) with characteristic features of airway macrophages such as high expression of 5-lipoxygenase (5-LOX), which resisted IL-4-mediated transcriptional repression. Exposure of aMDM to HDM resulted in the suppression of 5-LOX expression and product formation.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 393
In contrast, HDFM triggered increased protein production with thromboxane and prostaglandins D2 and E2 as major metabolites. HDFM also induced pro-inflammatory cytokines and chemokines in an overall M1-like inflammatory profile.

Finally, distinct changes in lipid mediator profiles occurred during the type 2 immune response to nediratam in mice. Conclusion: Our findings show that type 2 immune responses are characterized by fundamental reprogramming of the lipid mediator metabolism with macrophages representing particularly plastic responder cells. Targeting mediator reprogramming in airway macrophages may represent an approach to regulate pathogenic lipid mediator profiles in allergy or asthma. Funding: German Research Foundation (DFG), Else Kröner-Fresenius Stiftung

Poster Presentations

**P.CS.01.06**

**Hapten-induced contact hypersensitivity is upregulated in interleukin-19 knockout mice**

Y. Fujimoto, Y. Azuma;
Laboratory of Veterinary Pharmacology, Division of Veterinary Pharmacology, Graduate School of Life and Environmental Science, Osaka Prefecture University, Izumisano, Osaka, Japan.

Interleukin-19 is a member of the IL-10 family of interleukins and is an immuno-modulatory cytokine produced by the main macrophages. The gastrointestinal tissues of IL-19 knockout mice show exacerbation of innate immune cell infiltration in the issue. In vivo, there is an increasing focus on the interation and role of IL-19 with the function of T cells. Contact hypersensitivity (CHS) is a T cell-mediated cutaneous inflammation. Therefore, we asked whether IL-19 causes CHS. We investigated the immunological role of IL-19 in CHS induced by 2-fluorobenzoylfluorescein as a hapten. IL-19 was specially expressed in skin exposed to the hapten, and ear swelling was increased in IL-19 knockout mice. The exacerbation of the CHS response in IL-19 knockout mice correlated with increased levels of IL-17 and IL-6, but no alterations were noted in the production of IFN-gamma and IL-4 in the T cells of the lymph node. In addition to the effect on T cell response, IL-19 knockout mice increased production of inflammatory cytokines. These results show that IL-19 suppressed hapten-dependent skin inflammation in the elicitation phase of CHS.

**P.CS.01.07**

**The impact of gyclation on dendritic cell responses to proteins**


Introduction: Advanced Glycation End products (AGEs) are a group of protein modifications formed by the non-enzymatic reaction between a reducing sugar and a free amine group. AGEs are formed endogenously and for food, for example during dry roasting of peanuts. AGE-modified proteins, including dry-roasted peanuts, have an enhanced immunogenicity compared to unmodified proteins and induce a Th2 bias. However, dry-roasted peanut and other glycolated proteins do not induce conventional activation of dendritic cells (DCs). We hypothesized AGE-modified protein may instead induce chemo-attractant DC activation.

Materials and Methods: murine bone marrow derived DCs were pulsed with either dry-roasted or raw peanut proteins. AGE-modified egg allergens (hen egg lysozyme (HEL) or ovalbumin) or low dose lipopolysaccharide. Supernatants were collected to assess cytokine/chemokine secretion. DCs pulsed with AGE-modified HEL were also used for RNAseq.

Results: The transcriptomic and proteomic analyses demonstrated that modified proteins induced changes which were distinct from those elicited by the classical inflammatory stimuli lipopolysaccharide. There was a significant change in the expression of a small group of gene in response to HEL-Glucose. Pathway analysis using these results implicated a number of pathways, including those involved in the cell cycle and metabolism. Changes in secretion depended on both the modification type and the base protein.

Conclusions: Our results provide initial evidence for an alternative mode of DC activation by AGE-modified proteins. The variable secretory profiles induced by modified proteins indicate that multiple receptors are involved in these responses. Future studies will examine these potential receptors and their signalling pathways.

**P.CS.01.08**

**Complement drives IgE-mediated experimental food allergy through the C5a/C5aR1 axis**

A. Kordowski, A. T. Renickner, D. Wu, J. Lee, F. Wang, S. P. Hogan, J. Köhl, A. V. Wiese, T. Vollbrandt, Institute for Anatomy, Lübeck, Germany, Cincinnati Children’s Hospital and University of Cincinnati, College of Medicine, Cincinnati, United States.

Food-induced anaphylaxis is a serious allergic reaction caused by antigen cross-linking of IgE-loaded Fc-receptors on mast cells (MCs), leading to the release of pro-inflammatory mediators and disease manifestation. The exact mechanisms breaking oral tolerance and the effector pathways driving food allergy remain elusive. As complement activation occurs in food-induced anaphylaxis, we aimed to assess the role of C5a in disease pathogenesis. BALB/c wildtype (wt) and C5ar1-/- mice were subjected to oral antigen-induced food allergy model. Readouts included diarrhea development, changes in rectal temperature, hematocrit, antigen-specific serum IgE, MCP-1 and intestinal MC numbers as well as FcεR1-mediated MC functions including C5a receptor 1 (C5aR1) regulation. Further, histamine-mediated hypothermia and regulation of endothelial tight junctions was determined. Repeated oral OVA challenge resulted in diarrhea, hypothermia, increased hematocrit, high OVA-specific serum IgE and MCP-1 levels in wt mice. In contrast, mice C5ar1-/- were completely whereas female C5ar1-/- were partially protected from anaphylaxis development. The lower incidence of diarrhea in C5ar1-/- mice was associated with decreased OVA-specific serum IgE in male and mast cell activity (MCP-1 levels) in both sexes. Mechanistically, IgE-mediated degranulation and IL-6 production from C5ar1-/- BMDCs of both sexes was strongly reduced. Importantly, we show that strongly upregulated C5ar1 MC expression in vitro and in vivo. Finally, histamine treatment resulted in a milder temperature drop in C5ar1-/-mice than in wt mice. Our findings identify C5ar1 activation as an important driver of IgE-mediated food allergy. C5ar1 targeting may prove useful to suppress the inflammatory response in food-induced anaphylaxis.

**P.CS.01.09**

**Modulation of Csa receptor 2 expression in experimental allergic asthma**


C5aR2 (C5L2) regulates the allergic asthma phenotype. However its expression pattern on myeloid and lymphoid cells during the allergic effector phase is unknown. Recently, we generated and characterized a novel floxed tandem-dye Tomato fluorescent protein (tdTomato)-C5aR2 knock-in mouse, showing that C5aR2 expression is restricted to myeloid cells in the airways, the lungs and lymph organs. Using this reporter strain, we monitored C5aR2 expression during the effector phase of house dust mite-driven allergic asthma. C5aR2 reporter and wildtype mice developed an allergic phenotype with comparable airway resistance, mucus production, eosinophilic/neutrophilic airway inflammation and Th2/Th17 cytokine production. No major changes in C5aR2 expression occurred in myeloid cells during the allergic effector phase, in particular in airway eosinophils and a subset of neutrophils. However, we observed significant down regulation of C5aR2 on pulmonary macrophages. Lymphoid cells remained tdTomato-C5aR2 negative upon allergic inflammation except a small subset of NK cells, suggesting a functional role of C5aR2 in NK cells. In line with this observation, the NK cell marker Nkp46 was strongly upregulated in C5aR2-/- NK cells. In summary, we show that C5aR2 expression is not altered in the allergic effector phase on most myeloid cells. In contrast, C5aR2 is downregulated in pulmonary macrophages. Further, it controls Nkp46 expression in a subset of NK cells suggesting a novel function of C5aR2 in such cells during allergic inflammation. Funding DFG-RTG1911/A1

**P.CS.01.10**

**A critical role of IL-18 in the maturation of PD1L expressing pathogenic eosinophils**

A. Mishra, S. Upparahalli Venkateshao, A. Mishra;
Tufts University School of Medicine, New Orleans, LA, United States.

Eosinophils are multifunctional leukocytes with diverse functions in health and disease. We for the first time demonstrate that IL-18 has a critical role in the development and maturation of eosinophils. Herein, we provide evidence showing that IL-18 differentiates eosinophils in vivo, even in the absence of endogenous IL-5, both in vivo and under physiological conditions (in vivo). IL-18 and IL-5 differentiated ex vivo eosinophils have differences in size, shape, granularity and differentially regulated CD274 (PD1L) transcript expression. Most importantly, we evidence that IL-18 is critical for forming homeostatic eosinophils into mature PD1L1 expressing eosinophils. Notably, eosinophils with and without PD1L1 expression are present in healthy mouse and human blood. Moreover, the proportion of eosinophils that express PD1L1 is markedly increased in allergic mice and humans. Additionally, we report that all eosinophils in the lungs of asthmatic mice and the nasal lavage of asthmatic patients harbor PD1L1 expressing pathogenic eosinophils. Analysis of mouse and human eosinophil data in healthy and disease state indicates that IL-18 and IL-5 synergistically promote differentiation, maturation and proliferation of PD1L1 expressing pathogenic eosinophils in allergic disease states. Enhanced IL-18 expression is reported in almost all allergic diseases and our data suggest that it has a critical role in eosinophil differentiation and the maturation of PD1L1 expressing pathogenic eosinophils. Collectively, we first time identified the role of IL-18 in transforming naive eosinophils to pathogenic PD1L1 expressing eosinophils and this finding may have broad implications regarding non-invasive diagnostic and therapeutic strategies for eosinophil-related diseases.
Cytokine IL-33 is secreted by epithelial and endothelial cells during necrosis and stimulates humoral immune response. Elevated IL-33 expression in pulmonary epithelium of asthmatic patients correlates with exacerbation of allergen-induced inflammation and disease progression. We hypothesized that individual variations in the IL33 gene expression may be explained by polymorphisms of non-coding regulatory regions, in particular by SNP rs928413 located in an promoter region in the IL33 locus and associated with development of asthma.

Activities of the IL33 promoter variants containing different rs928413 alleles were assessed upon transfection of the corresponding luciferase reporter constructs into NCI-H292 pulmonary epithelial cell line followed by TNFα stimulation. CREB1 binding to DNA was estimated using pull-down assay.

We observed that rs928413 risk allele creates a functional binding site for CREB1, a transcription factor that stimulates expression of a number of inflammatory mediators. IL33 promoter containing active CREB1-binding site showed significantly elevated activity in stimulated human lung carcinoma cells.

Our data suggest that differential binding of the rs928413 alleles to may underlie the emerging asthma phenotype in response to increased IL33 gene expression and of systemic inflammation.

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Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

396

P.C.05.16
The mechanism of reactive oxygen species-induced apoptosis in mast cells in response to lysosomotropic agents
A. Polvand1, F. R. Meis2, G. Peijler1,2
1Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, 2Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Mast cells are infamous for their detrimental role in allergic reactions and inflammatory lung diseases, such as asthma. Therefore, efficient strategies to limit their adverse effects in such pathological settings are needed. We have previously reported that lysosomalotropic agents, such as mefloquine, induce mast cell apoptosis through granule permeabilization and reactive oxygen species (ROS) induced production of reactive oxygen species (ROS) in mast cells. Here we sought to explore the underlying mechanism of ROS-induced apoptosis in mast cells upon mefloquine treatment. Interestingly, we found that the ROS production in response to mefloquine is diminished in mast cells deficient in serpin C proteoglycan, suggesting that mast cell granules (also known as secretory lysosomes) may play a role in the ROS production. Given that previous reports highlighted a role for lymosom al metal ions in ROS production, we next pre-incubated mast cells with chelators of iron (deferoxamine) and/or copper (tetra-ammonium-clotolate), followed by mefloquine treatment and assessment of ROS levels. We observed that deferoxamine or tetra-ammonium-clotolate-treated mast cells generated reduced levels of ROS after mefloquine treatment, indicating that iron and copper ions are key component in mefloquine-induced ROS production in mast cells. Another potential source of ROS could be through the action of NADPH oxidase. However, our preliminary data show that inhibition of NADPH oxidase had little inhibitory effect on the ROS production in response to mefloquine, arguing against this possibility. Taken together, our preliminary data suggest that enhanced ROS production upon mefloquine treatment is likely the result of the Fenton reaction to a large extent.

P.C.05.17
A fragment of extracellular matrix collagen drives epithelial remodelling and airway hyper-responsiveness in allergic airways disease
D. F. Peters1, T. Peiron2, A. Shoemark3, S. Akhtar4, S. A. Walker5, A. Gaggi6, G. Tavernier7, L. G. Gregory5, A. Simpson8, C. M. Lloyd5, R. J. Slennige5
1Department of Comparative Medicine, Interuniversity Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University Vienna, Vienna, Austria, 2Department of Cell Biology and Genetics, Faculty of Science, Palacky University, Olomouc, Czech Republic, 3Center for Plant Biotechnology and Genomics and Department of Biotechnology-Vegetal Biology, ETISIAB, Technical University of Madrid, Madrid, Spain, 4Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectology and Immunology, Medical University Vienna, Vienna, Austria.

Introduction: Our in silico calculations predicted that the major allergen from birch, Bet v 1, has lipocalin-like function and is able to bind iron via high-affinity iron-chelators called siderophores. Only when loaded with iron it was in vivo able to prevent the development of IgE and allergic sensitization. Here we analyzed in vitro the iron-binding capacity of Bet v 1, using flavonoids (catechol-type-siderophores). Furthermore, we demonstrated the bioactive function of these iron-chelators focusing on the PGP. Materials and Methods: UV/Vis-spectra of three major flavonoids (Quercetin, Catechin, Epi-Catechin) in the presence or absence of allergens and iron were analyzed. Using the reporter cell line AZ-AhR activation of the AhR-pathway was determined by measuring luciferase activity. Presence of iron was measured with Calcein.

Results: Our data suggests that the major allergen Bet v 1 is involved in the generation of allergens in the presence of allergens and iron were analyzed. Using the reporter cell line AZ-AhR activation of the AhR-pathway was determined by measuring luciferase activity. Presence of iron was measured with Calcein.

Conclusion: Flavonoids act as siderophores and bind to Bet v 1. Only the loaded form of Bet v 1 significantly stimulated AhR-activation via active transport of these flavonoids and iron into the cell, thereby enabling an immune-suppressive stimulus. The ligands of allergens may thereby be decisive for the subsequent immune response promoting tolerance.

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P.C.05.18
Active transport of iron-flavonoid complexes by the major allergen Bet v 1 leads to enhanced activation of the aryl hydrocarbon receptor
1Department of Comparative Medicine, Interuniversity Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University Vienna, Vienna, Austria, 2Department of Cell Biology and Genetics, Faculty of Science, Palacky University, Olomouc, Czech Republic, 3Center for Plant Biotechnology and Genomics and Department of Biotechnology-Vegetal Biology, ETISIAB, Technical University of Madrid, Madrid, Spain, 4Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectology and Immunology, Medical University Vienna, Vienna, Austria.

Introduction: Our in silico calculations predicted that the major allergen from birch, Bet v 1, has lipocalin-like function and is able to bind iron via high-affinity iron-chelators called siderophores. Only when loaded with iron it was in vivo able to prevent the development of IgE and allergic sensitization. Here we analyzed in vitro the iron-binding capacity of Bet v 1, using flavonoids (catechol-type-siderophores). Furthermore, we demonstrated the bioactive function of these iron-chelators focusing on the PGP. Materials and Methods: UV/Vis-spectra of three major flavonoids (Quercetin, Catechin, Epi-Catechin) in the presence or absence of allergens and iron were analyzed. Using the reporter cell line AZ-AhR activation of the AhR-pathway was determined by measuring luciferase activity. Presence of iron was measured with Calcein.

Results: Our data suggests that the major allergen Bet v 1 is involved in the generation of allergens in the presence of allergens and iron were analyzed. Using the reporter cell line AZ-AhR activation of the AhR-pathway was determined by measuring luciferase activity. Presence of iron was measured with Calcein.

Conclusion: Flavonoids act as siderophores and bind to Bet v 1. Only the loaded form of Bet v 1 significantly stimulated AhR-activation via active transport of these flavonoids and iron into the cell, thereby enabling an immune-suppressive stimulus. The ligands of allergens may thereby be decisive for the subsequent immune response promoting tolerance.

The study was supported by the Austrian Science Fund FWF, grant SFB F4606-B28 to EJ.

P.C.05.19
Olaparib, a Poly(ADP-ribose) Polymerase Inhibitor Abates Ovalbumin-induced Airway Inflammation and Remodeling in Murine Model of Chronic Asthma
G. S. Seth1, A. S. Narsa2
1Department of Biochemistry, Panjab University, Chandigarh, India.

Poly(ADP-ribose) polymerase (PARP) has been reported to play a crucial role in the manifestation of allergen-induced airway inflammation, a characteristic feature of acute asthma. However, the role of PARP in airway remodeling (a hallmark of chronic asthma), is not completely known. Accordingly, the present study was designed to evaluate the potential of olaparib (a PARP inhibitor) on airway remodeling traits using an ovalbumin (OVA)-induced murine model of chronic asthma. The results demonstrated that olaparab administration (5 mg/kg b.wt. via i.p., 30 minutes after every OVA challenge for six-weeks attenuates the airway inflammation, mucus production, collagen deposition in airways and the expression of coupled factors such as TGF-β, Muc5ac, Col1α1, MMP-9, and TGF-β in lung tissues. Additionally, the OVA-induced alteration in the level of ROS, MDA, and protein carbonyls was significantly reduced in olaparib treated groups as compared to the control group. Subsequently, we show that high amounts of ROS generated in response to OVA challenge was significantly reduced in olaparib treated group. Treatment with olaparib (5 mg/kg b.wt.) also attenuated the airway remodeling traits in the OVA-challenged group. Additionally, the expression of LTA4H was significantly reduced in olaparib treated group as compared to the control group. These results suggest that olaparib, a PARP inhibitor, may be a potential therapeutic agent in the treatment of chronic asthma.

P.C.05.20
Allergic sensitization profile of polysensitized asthmatic patients in Southern China using molecule-based IgE technique
H. Hsu, B. Sun, W. Luo
First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China.

Objective: Most of allergic asthma patients are sensitization to a variety of allergens. This study aimed to analyze the sensitization profiles of polysensitized asthmatic patients in southern China utilizing molecule-based IgE diagnostic technique. Method: Serum samples from 63 asthma patients in southern China were tested with SACC for specific immunoglobulin E (sIgE) against 112 single allergen components. Results: In this group of patients, 79.36% showed sIgE positive to more than three allergen components. Polysensitized asthmatic patients in southern China were mainly allergic to dog f2 (68.25%), dog f1 (66.67%), dog p 1 (65.08%), pollen p 2 (61.90%), and cat f 1 (26.98%), cat s 1 (15.87%), dog p 4 (14.29%), cat s 1 (12.70%), dog r 10 (11.11%) and dog r 2 (11.11%). Polysensitized asthmatic patients complicated with rhinitis showed higher positive rates of the allergen components dog f 4 (19.05% vs. 0.00%), cat s 1 (24.79% vs. 20.69%), dog r 10 (0.00%) and dog r 2 (21.95%) than patients without rhinitis (P<0.001). Among food allergen components, the walnut allergen component nlgk 2 showed the highest positive rate (95.2%). An optimal scaling analysis indicated that a positive test of dog r 10 was associated with food allergy (Cronbach’s Alpha = 92.0%). Conclusions: The sensitization profiles of polysensitized asthmatic patients in southern China were different from other countries. Polysensitized asthma patients complicated with rhinitis showed higher positive rates for dog p 4 and cat s 1 than without. Polysensitized asthmatic patients who positive for dog r 10 were associated with food allergy.
POSTER PRESENTATIONS

P.CS.01.21
The value of allergy screening in Chinese adult patients with chronic respiratory diseases
H. Hu, B. Sun, P. Zheng;
First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China.
Objective: The prevalence of allergy-induced chronic respiratory disease (CRD) is increasing annually. This study aimed to analyze sensitization characteristics of adult Chinese CRD patients and the value of allergy screening. Methods: Serum immunoglobulin E (IgE) was detected with an allergy screening test. Total immunoglobulin E (IgE) levels were measured in 85 asthma patients, 98 chronic obstructive pulmonary disease (COPD) patients, and 69 patients with other CRDs. Results: The total positive rate of allergy screening among CRD patients was 36.3%. Asthma had the highest rate (45.9%), followed by COPD (32.7%) and other CRDs (29.0%). The positive rate of allergy screening was significantly higher among urban asthma patients (56.1%) than among rural patients (25.0%, P < 0.05), and significantly higher among office staff (68.9%) than among outdoor workers (42.8%, P < 0.05).

P.CS.01.22
Maternal IgE impairs the maturation of offspring intrathymic IL-17-producing yδT cells: possible implications for maternal and human allergy
M. G. de Oliveira, A. A. Lira, F. R. Sgnotto, A. H. Inoue, L. S. Santos, A. J. Duarte, M. Leite-de-Moraes, J. R. Victor;
1School of Medicine - USP, Sao Paulo, Brazil, 2Institut Necker-Enfants Malades (INEM), Paris, France, 3 Laureate International Universities (FMU), Sao Paulo, Brazil.
Using a well-standardized murine model of offspring allergy-mediated by maternal allergen immunization, we aimed to evaluate the relationship between IL-17-producing yδT cells and allergy inhibition by focusing on the regulation of the intrathymic maturation and yδT cell biology. Female mice were immunized or not, and the allergic response, frequency of yδT cell subsets and cytokine production of the offspring were analysed by flow cytometry. The effects of passive in vivo transfer of thymocytes or purified IgE were investigated in offspring. A translational approach was employed to analyse yδT cells in the thymus and PBMCs from humans. Maternal immunization reduced the frequency of spontaneous IL-17-producing yδT cells in the thymus, spleen and lung of offspring. This effect was mimicked by the in vivo treatment of females with purified IgE. yδT cells directly interacted with yδT cell membranes. Human infant intrathymic yδT cells showed reduced IL-17 production in response to purified IgE from non-atopic individuals, whereas adult peripheral yδT cells from atopic individuals were prone to produce IL-17 in response to IgE. Together, our results reveal that IgE from atopic mice can influence the thymic yδT cell maturation. Further, IgE is an unprecedented modulatory factor of murine and human yδT cells.

P.CS.01.23
Mango chitinase is a major allergen in Chinese pediatric patients
1College of Medicine, Shenzhen University, Shenzhen, China, 2Clinical molecular diagnostic laboratory, Shenzhen Children’s Hospital, Shenzhen, China, 3Department of Pediatrics, Third Affiliated Hospital of Sun Yat-sen University, Shenzhen, China.
Introduction: Allergies to mango are frequently observed in clinical practice. However, only one allergen has been identified from mango until now. Materials and Methods: Protein crude extract of mango flesh was resolved by using 1-D SDS-PAGE. The reactive bands were analyzed by MS/MS mass spectrometry. A band showed significant homology to the mango chitinase (Genbank Accession: ACD69683.1). The cDNA of the chitinase was synthesized and cloned into a plasmid pMAL-C5X and expressed as a recombinant protein. The recombinant protein towards mango allergens was assayed by using western blot. The linear IgE epitopes of the chitinase were then analyzed by six peptides spanning the entire protein with putative potential of IgE reactivity. Results: The mango chitinase reacted with IgE of 9 out of 13 sera (69%) with mango allergy. Only one peptide (8-28) which contains the chitin-binding domain possesses IgE reactivity. Conclusions: The mango chitinase is a major allergen in Chinese pediatric patients.

P.CS.02.02
Cyclic-dipeptide isolated from Hirsutella sinensis mycelium attenuates ovalbumin-induced asthma in a murine model
1Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan, 2Microbiota Research Center, Center for Molecular and Clinical Immunology, Chang Gung University, Taoyuan, Taiwan, 3Graduate Institute of Natural Products, Chang Gung University, Taoyuan, Taiwan, 4Chang Gung Biotechnology Corporation, Taoyuan, Taiwan.
Background: Asthma is a chronic inflammatory disorder that affects millions of people worldwide. It is characterized by eosinophilic inflammation, airway hyperresponsiveness, and airway remodeling. Traditional Chinese Medicine has been used for centuries for their immunomodulatory and anti-inflammatory effects. Hirsutella sinensis, a medicinal mushroom, was found to have anti-asthmatic effects. Water extracts from Hirsutella sinensis mycelium reduced ovalbumin-induced asthma and TH2 responsiveness in mice, yet the bioactive compound that promotes this occurrence remains unknown.
Materials and Methods: Bioactivity-graded fractionation such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and an in vitro TH2 cell inhibition screening model was used to isolate and identify the cyclic-dipeptide from Hirsutella sinensis mycelium. Ovalbumin-induced asthma model in BALB/c mice were utilized to test the compound in vivo.
Results: Cyclic-dipeptide directly inhibited TH2 responsiveness by decreasing production of TH2-associated cytokines such as IL-4, IL-5, and IL-13 in vitro and in vivo. Further in vivo asthma model demonstrates that cyclic-dipeptide from Hirsutella sinensis mycelium dose-dependently attenuates ovalbumin-induced asthmatic responses, including airway hyperresponsiveness, immune cells infiltration, and cytokine responses. Conclusions: Taken together, we identified a potent anti-asthmatic and anti-allergic compound isolated from HSM and potentially explore its underlying mechanisms.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Our findings suggest that the therapy with autologous activated T-cells led to an improvement in the quality of life in patients with BA compared before treatment. That was indicated the efficacy of the immunotherapy. Observed significant improvement in the “activity limitations” domain compared with the values before the beginning of therapy. Also there was a tendency (p=0.08) to increase carried out using the AQLQ(S). The AQLQ(S) has 4 domains: activity limitations, symptoms, emotional function, and exposure to environmental stimuli. After 2 months, it was approved by the ethical commission of RIFCI. After obtaining Informed Consent to participate in the study, patients were injected subcutaneously with autologous activated T-cells. Today bronchial asthma (BA) is one of the most common chronic diseases associated with immunological disorders. The purpose of this study was to study the effect of immunotherapy with autologous activated T-lymphocytes, including the evaluation of the quality of life in the dynamics of this type of therapy in patients with BA.

Introduction: Asthma is a complex chronic inflammatory disease characterised by airway inflammation, remodelling and hyperresponsiveness (AHR). Members of the AP-1 family of transcription factors have been shown to play important roles in the activation of the immune system and the control of cellular responses. Here we have investigated the role of the known AP-1 family member, Fra2 in experimental asthma.

Methods and Results: Gene expression profiling of Fra2 overexpressing (TG) mice revealed a high number of regulated genes associated with airway remodelling, inflammation and mucus production. In line with this finding TG mice exhibited increased airway remodelling, with peribronchial collagen and smooth muscle thickening as well as mucus production. TG mice possessed a strong inflammatory infiltrate in the lung, predominately, eosinophils and T cells and increased expression of Th2 cytokines and eotaxin. Furthermore, TG mice possessed AHR in response to increasing doses of methacholine. Therapeutic intervention via IL-13 blocking antibodies or corticosteroids partially reduced AHR, mucus secretion and eosinophil infiltration. However, only corticosteroid treatment could reduce all aspects of airway remodelling. Conclusion: Here we have demonstrated a novel model of non-allergic asthma, which does not require the application of exogenous allergens. Fra2 represents a key molecule that coordinates several aspects of asthma pathogenesis, including airway inflammation, remodelling and hyperresponsiveness. This study was funded in part by the Austrian Science Fund (FWF): P27848-B28 and Austrian National Bank grant number 16187.
Abstracts of the 5
POSTER PRESENTATIONS

p<0.05), IL-13 (75.8±8.7%, p<0.001) and IL-10 (80.8±13.1%, p<0.001) after 48 hours. After 72 hours, BX-795 also reduced secreted IL-5 and TNF-α levels.

BX-795 is an inhibitor of 3-phosphoinositide-dependent kinase-1 (PDK1) and TANK-binding kinase-1 (TBK1), involved in T cell receptor (TCR) and innate signaling. We investigated

Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria.

P. A. Tauber

P .C5.02.19

Anaphylaxis associated with influenza vaccines in patients without egg allergy was very unlikely. We reported a pediatric case of influenza vaccine-associated anaphylaxis without

D. Shim

P .C5.02.18

Institut de Pharmacologie et de Biologie Structurale, Toulouse, France.

P. Schmitt

P.C5.02.17

IL-9 is a key driver of chronic and allergic inflammation at mucosal surfaces, with important roles in the activation and survival of mast cells and ILC2s. IL-9 reporter mice have established T cells (Th9) and ILC2s as major sources of IL-9 in vivo. The discovery that TGF-β and IL-4 cooperate for induction of Th9 cells has been an important breakthrough.

However, the signals that induce IL-9+ ILC2s remain incompletely characterized. Here, we show that IL-33 is a critical activator of ILC2s, with important roles in type-2 innate immunity and allergic inflammation (Cayrol, Duval and al., Nature Immunology 2018). The discovery that TGF-β and IL-4 cooperate for induction of Th9 cells has been an important breakthrough.

P. Schmitt

P.C5.02.16

Profile of component-resolved diagnostics allergy by microarray assay in a southern Spanish area

M. San Jose-Casan, M. Vilches-Moreno, D. Garcia-Cuesta, A. Sampaolo;

UGC Hematology, Immunology and Genetics, Hospital Universitario Puerta del Mar, Cadiz, Spain.

Identification of sensitization profiles in our geographic area by analysis of component-resolved diagnosis allergy in multiplex support for detection of relevant allergen sources, differentiation between genuine and cross-reactive reactions, and analysis of risk and severity of allergic reactions.

160 Patients (72 male/88 female), median aged 31.3 (SD 16.4, range 2-74 years) with suspected allergic symptoms were selected for testing IgE antibodies by microarray assay (ISAC). 74% of the patients showed two or more clinical manifestations at respiratory, digestive and skin levels. 26% of the patients studied showed only one symptom. Unexplained anaphylaxis was present in 62 patients.

ISAC analysis showed sensitization in 131 patients (82%). Sensitization were not found in 29 cases (18%). 87% patients (54%) were positive for both, genuine and cross-reactive allergens. Main specific primary sensitization components in respiratory allergy were: ole e 1 (56%), Derp1 (33%), Derp2, Phlp1 (37%), Phlp5 (8%), Alt a 1 (17%), Salk1 (13%), Art v1 (7%). Primary sensitization components in digestive component were: jug g2 (15%), jug g1 (11%), Arah1 (4%) and Cora9 (5%) and fruits: Pru p1 (5%), Act d1 (6%). Main reactions detected in protein family were: storage proteins (47%), lipocalins (21%), Tropomyosins (16%), Profilins (11%), PR10 protein (7%) and polcalcin (3%).

The cohort consisted of 51 patients that showed that the only significant risk factor for BPT occurrence (OR: 7.2, p<0.007). Fourteen subjects were included for blood analysis. The amounts of IL-9 in unstimulated sera were significantly lower that in patients who had BPT than controls. The BH cut-off that best identified a history of BPT was 175.31 (sensitivity: 62.5%, specificity: 100%). Following the stimulation, BH reduced compared to the unstimulated condition and the difference between groups remained significant (p<0.05).

Our study is the first that low levels of bloemycin hydrolyze in allergic individuals might be predisposing to a possible pathway of inflammation. This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK). (Grant No: 1165019).

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Interestingly, Th17 cytokines were not reduced while IL-2 was strongly increased peaking at 72 hours ([4.7±1.4-fold, p<0.001]) in murine cells. Recent studies have shown that the activation of mast cells via antigen-mediated triggering of FcεRI causes immediate anaphylactic reactions. Subsequent mechanistic investigations revealed that in two different FcεRI-mediated animal disease models of passive systemic and passive cutaneous anaphylaxis, Lyst-mutant Bg-J mice display substantially reduced immediate anaphylactic reactions. This study was funded by the Longfonds.

Our findings indicate that the IgG4 subclass can redirect pro-allergic M2a macrophages to an M2b-like immunosuppressive phenotype. This may suggest the involvement of macrophages in tolerance induction in AIT. Supported by the Austrian Science Fund (FWF) projects DK W1248, SFB-F4609 and the Medical University of Vienna.

We here report that in two different FcεRI-mediated animal disease models of passive systemic and passive cutaneous anaphylaxis, Lyst-mutant Bg-J mice display substantially reduced immediate anaphylactic reactions. This study was funded by the Longfonds.

Future experiments will reveal the molecular mechanisms underlying our observations and will clarify the Th2 blocking potency of BX-795 in allergen-specific asthma models in vivo. Supported by the Austrian Science Fund (FWF) projects DK W1248, SFB-F4609 and the Medical University of Vienna.

Supported by FWF-project SFB F4606-B28 to EJJ.

In conclusion, BX-795 attenuates IgG4 mediated pro-allergic M2a to an immunosuppressive M2b phenotype and may represent a new treatment option for IgG4-mediated allergies. Future studies will clarify the Th2 blocking potency of BX-795 in allergen-specific asthma models in vivo. Supported by the Austrian Science Fund (FWF) projects DK W1248, SFB-F4609 and the Medical University of Vienna.

In summary, our data demonstrate a negative tissue-dependent regulatory role of Lyst in immediate allergic reactions and indicate a role during de novo secretion in mast cells. This study was funded by the Longfonds.

Role of the membrane trafficking regulator Lyst during type 1 allergic responses

We here report that in two different FcεRI-mediated animal disease models of passive systemic and passive cutaneous anaphylaxis, Lyst-mutant Bg-J mice display substantially reduced immediate anaphylactic reactions. This study was funded by the Longfonds.

In summary, our data demonstrate a negative tissue-dependent regulatory role of Lyst in immediate allergic reactions and indicate a role during de novo secretion in mast cells.
Allergenic potential of aquaculture fish due to the presence of Anisakis sp L3 proteins

M. Corballes Gonzalez1, A. Rojas Gomez1, I. Sánchez Alonso2, M. Coreche Recacoechea3, S. Cabaco Arcos1, A. Navas Sánchez1, S. Pascual del Hierro1, M. González-Muñoz1; 1Foundation for Biomedical Research Hospital La Paz, Paseo de la Castellana, 28046 Madrid, Spain, Madrid, Spain, 2State Agency Superior Council of Scientific Research, CSIC, Madrid, Spain, Madrid, Spain, 3State Agency Superior Council of Scientific Research, CSIC, Madrid, Spain, Madrid, Spain.

Some feed components can be carried-over into and detected in animal products. The aim of this work was to detect Anisakis sp. antigens/allergens in aquaculture fish due to carry-over from fish feed. We have analyzed 4 aquaculture fish (Scophthalmus maximus) and 2 fish feed collected in Spanish farm. Protein extracts from fish and feed were prepared and the presence of parasite allergens was assessed by immunoblotting using anti-Ani s 4 antisera and a pool of Anisakis allergic patients’ sera. Allergens were identified by sequencing analysis and basophil activation test was performed to assess if the allergens were functionally active. Allergens were detected in both fish and feed extracts with anti-Ani s 4 and patient’ sera. In addition, both extracts were able to activate basophils. The presence of Ani s 4 was confirmed by immunoblotting inhibition and specific peptides of Anisakis sp. haemoglobin, enolase, Ani s 9 were identified by mass spectrometry. Aquaculture fish contain Anisakis sp. allergens and can pose an unsuspected source of parasite allergens. The presence of allergens of Anisakis sp. in feed suggests that these allergens are carried-over from feed.

Regulatory T cells in cord blood of children of allergic and healthy mothers

V. Čerňová1, O. Novotná1, P. Petrášková1, K. Machálovičová1, K. Barková1, L. Prokeliová1, J. Hrdý1; 1Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University, Prague, Czech Republic, 2Institute for the Care of Mother and Child, Prague, Czech Republic.

Allergic diseases represent a major issue in both clinical and experimental immunology. Despite intensive research, allergic status of the mother remains the strongest universally accepted predictor of increased risk of allergy development.

Disregulation of balance among branches of immune response, chiefly an excess of Th2 polarization, is a key underlying cause of allergic diseases. Regulatory T cells (Treg) are crucial for maintaining the correct balance and inducing tolerance towards allergens. Function of Treg may therefore contribute to increased risk of allergy.

We studied T cord blood in children of healthy mothers (with lower risk of allergy development) and allergic mothers (with relatively higher risk) by flow cytometry, aiming to find markers which could help predict allergy development. We observed higher percentage of Treg in cord blood of high-risk children compared with lowrisk group. However, expression of several markers of Treg function (intracellular IL10 and TGF-β, MFI of FoxP3, PD1) was decreased in Treg of high-risk children. These results may be explained by an expansion of Treg population trying to compensate for inadequate regulatory capacity.

We attempted to further characterize functional activity of Treg directly (inhibition of proliferation of CFSE stained target cells) and by expression analysis of selected genes (qPCR).

Using flow cytometry we also analyzed expression of Helios, a transcription factor originally considered specific for thymic-derived Treg, which may also be upregulated during T cell activation.

Work supported by AVZ CR 15-26587A and Charles University research programmes Progres Q25/LF1 and SVO 296 369.

Cytokine patterns and impaired cytotoxic activity of NK cells in children with atopic dermatitis

E. Cetin Aktas1, N. Akdeniz2, B. N. Melgert3, T. A. van der Veen3; 1Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey, 22Marmara University , Research and Training Hospital, Division of Pediatric Allergy and Immunology, Istanbul, Turkey, 3University of Groningen, Groningen, Netherlands, Groningen, Netherlands.

Atopic dermatitis (AD) is the most common, chronic, and pruritic inflammatory skin disease skin disease, and primarily affects children. The role of NK cells in development of this disease is still poorly documented. The current study was undertaken to determine NK1.1, NK2 and NK regulatory cytokine profiles. The expression of activating receptors as well as the cytotoxic activity of NK cells in children with AD. The study group consists of atopic (n=10), non-atopic (n=6) and healthy subjects (n=13). Patients were multisensitized and had high serum total IgE levels. Cytotoxic capacity, expression of CD56 brightly and CD56 dim NK cell subsets, NKp46 and NKp44 expressions, and intracellular IL-4, IL-10 & IFN-γ levels were determined by flow cytometry. The percentages of CD3+CD16+CD56 dim NK cells were significantly diminished in atopic patients compared to healthy subjects, CD56 brightly NK cell subset was significantly increased in non-atopic children than atopic group. In contrast to diminished cytotoxic capacity, expression of CD56 brightly and CD56 dim NK cells, increased expression of activatory receptor NKp46, CD56+CD3+IL-4 and CD56+CD3+IL-10+ NK cells in whole AD children compared to those with healthy subjects were obtained. High percentages of IL-4 and IL-10 producing NK cell subsets demonstrating an Th2 and regulatory type cytokine tendency of NK cells in AD patients. Our findings suggested impaired NK cell functions in AD patients implying high activation, accompanied by decreased cell cytotoxicity, which would contribute to the pathogenesis of disease and partially explain the tendency to the skin viral infections observed in those patients.

Toll-like receptor 2 ligations enhances therapeutic effects of mesenchymal stem cells on murine model of asthmatic inflammation

H. Yu, Y. Chen, B. Chiang; Graduate Institute of Clinical Medicine, Taipei City, Taiwan.

Introduction: Under current therapeutic strategies, the cure to asthma remains elusive; thus novel approaches for treating asthma are desperately needed. Despite that mesenchymal stem cells (MSCs) have recently been established as potential candidates by virtue of their immunomodulatory properties, the underlying heterogeneity of MSCs dictates their therapeutic efficacy.

Materials and Methods: Here we evaluated a toll-like receptor (TLR) 2 ligation protocol of MSCs to augment their therapeutic efficacy on asthma.

Results: We surmise that a TLR2 ligand, Pam3CSK4, enhanced the therapeutic effects of MSCs on asthmatic inflammation in mice at low concentration (1 μg/mL) for a longer induction period (96 h) in a post-treatment manner. We further validated this regimen by demonstrating that Pam3CSK4 activated STAT3 in BM-MSCs through IL-6, which was likely stimulated with NF-κB signaling. NO, the key suppressive molecule of Pam3CSK4, grimed BM-MSCs, was later highly increased through upregulation of iNOS, which was in the downstream of STAT3 phosphorylation. The intensified suppressive functions of BM-MSCs were then executed by inducing CD4+CD25+Foxp3+ regulatory T cells in a post-treatment manner.

Conclusion: The results demonstrated that TLR2 ligand, Pam3CSK4, could modulate the function of BM-MSCs and alleviate airway inflammation. The consistent therapeutic outcomes and the valid suppressive mechanisms in the study might shed light on the eliminating proinflammation-prone uncertainties of MSCs, and enhancing the future feasibility of obtaining long-lasting effects with this regimen.

Are eosinophils major contributors to oxidative stress during asthma exacerbations?

L. E. S. de Groot1, Y. S. Sabogal Piñeros2, S. M. Bal1, M. A. van de Poel1, W. Kulk1, T. A. van der Veen1, B. N. Melgert1, F. O. Martinez2, J. Hamann1, R. Lutter1; 1Academic Medical Center, Amsterdam, Netherlands, 2University of Groningen, Groningen, Netherlands, 3University of Surrey, Guildford, United Kingdom.

Introduction: Asthma exacerbations are predominantly triggered by respiratory viral infections and characterized by eosinophilic airway inflammation and increased oxidative stress. Eosinophils can produce reactive oxygen species (ROS) and a link between eosinophils and oxidative stress during exacerbations is thus likely. Attenuation of eosinophils using anti-IL-5 (Mepolizumab) in severe asthmatics significantly reduces exacerbation rates and corticosteroid dependency. Yet, the impact of reduced eosinophils on ROS production has not been investigated so far. Therefore, we aimed to study the contribution of eosinophils to oxidative stress during virus-induced asthma exacerbations.

Methods: Mild asthmatics received one high dose of Mepolizumab or placebo and after two weeks were subjected to rhinovirus 16 (RV16) infection. Exhaled breath condensate were collected before and after RV16 and levels of malondialdehyde (MDA), asymmetric dimethylarginine, nitrotyrosine, bromotyrosine, chlorotyrosine and dityrosine were measured using ultra-performance liquid chromatography-tandem mass spectrometry.

Results: Mepolizumab effectively attenuated eosinophil numbers in the circulation and locally in the airways. Mepolizumab effectively attenuated eosinophil numbers in the circulation and locally in the airways. Mepolizumab significantly reduced RV16-induced MDA levels and nitrotyrosine in the RV16-infected group. Mepolizumab could modulate the function of BM-MSCs and alleviate airway inflammation. The consistent therapeutic outcomes and the valid suppressive mechanisms in the study might shed light on the eliminating proinflammation-prone uncertainties of MSCs, and enhancing the future feasibility of obtaining long-lasting effects with this regimen.

This work is supported by the Lung Foundation Netherlands (4.115.002 and 3.2.10.069) and GIS (CRT 114696).
We demonstrated that lower vitamin D level was noted in the breast milk of mother having baby with infantile atopic dermatitis. Analysis of breast milk at 2-month post-partum showed that no significant difference between two groups. However, Vitamin D level in the breast milk for healthy infants was higher than in 2 and 6 month postpartum and the mothers are interviewed by the dietician for the diet questionnaire (diet pattern analysis) at the same time. Results: We enrolled 87 infants, of whom 28 were healthy and 59 patients with allergic disease. The breast milk vitamin D level in the main group was 28.6±5.6 vs. 0.7±0.2, P<0.01, as were circulating anti-HDM IgG1 levels (0.7±0.15 vs. 0.26±0.01, P<0.01). Interestingly, the pattern of cytokine-producing cells was clearly distinct in the lungs of asymptomatic NOD mice as compared to BALB/c. First, the proportions of IL-4, IL-13 and IL-5 producing CD4+ T cells were increased (13.2±3.1 vs. 6.7±1.9, 9.1±1.0 vs. 2.7±0.2, and 5.9±1.1 vs. 2.0±0.3 respectively, all P<0.01). Secondly, CD4+ T cells were the major IL-5 producers in NOD mice (60.8±4.0% TCRβ4+CD4+ among IL-5+ cells) while in BALB/c the source of IL-5 were type 2 ICS (60.6±6.7% TCRβ17+). Third, an exacerbated production of IL-17 was found in NOD mice which sources included conventional CD4+ T cells (3.1±0.3 vs. 1.1±0.2, P<0.01), CD8+ T cells (Il22+ T cell 11.2±1.6 vs. 1.6±0.1, P<0.01) and IL-17-producing Vγ9Vδ2+ T cells (2.8±0.4 vs. 0.9±0.04, P<0.05). Our results demonstrate that the NOD strain develops a unique form of severe allergic-induced asthma associated with exacerbated TH2 and IL-17-biased immune responses.

**P.C5.03.07**

Analysis of vitamin D level and reduced mast cell degranulation by single-stranded oligonucleotides

A. Dondašá, A. Rönnberg1, H. Ma2, S. Nilsson3, E. Magnusdottir4, L. Adam5, G. Nilsson6, M. Lagerström7, A. Spetz8

1Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm, Sweden, 2Immunology and Allergy Unit, Department of Medicine, Karolinska Institute, Salo, Sweden, 3Department of Neuroscience, Uppsala University, Uppsala, Sweden.

Introduction: Itch is a large problem in numerous skin disorders. The Mas-related G protein-coupled receptor X2 (MRGPRX2) has been shown to modulate itch by inducing non-IgE-mediated mast cell degranulation and the release of endogenous inducers of pruritus such as histamine. Various basic cationic substances, including inflammatory peptides and lipids, can trigger MRGPRX2 and thereby induce pseudo-allergic reactions characterized by histamine release and inflammation. Here, we investigated the capacity of an immunomodulatory single-stranded oligonucleotide (ssON) to inhibit itch and mast cell degranulation. Materials and Methods: To determine the effect of ssON on itch, we performed behavioral studies in established mouse models and collected skin biopsies. We examined the effect of ssON on MRGPRX2 activation in vitro by measuring degranulation (flow cytometry, ELISA) in a human mast cell line and calcium influx in MRGPRX2-transfected cells. Results: We observed that intradermal injection of ssON in mice was able to inhibit itch induced by the basic secretagogue C48/80 in a dose-dependent manner. Evaluation of histological staining at the injection site revealed that ssON appeared to inhibit mast cell degranulation in murine skin treated with C48/80. ssON also demonstrated the capacity to inhibit MRGPRX2 activation in vitro by blocking mast cell degranulation and calcium influx in MRGPRX2-transfected cells induced by certain basic secretagogues in a dose-dependent manner. Conclusions: ssON can inhibit IgE-independent mast cell degranulation and itch. Since there is a need for small molecules to block MRGPRX2-mediated activation of mast cells, ssON could be utilized to ameliorate itch in certain pathological settings.

**P.C5.03.08**

The non-obese diabetic mouse as a novel model for severe allergic asthma: the respective roles for T, 2, and IL-17-producing cells

A. Foray1,2, C. Marquet2, C. Pequet2, C. Dietrich2, F. Machovina2, M. Dyli3, J. Bach2, L. Chatenoud2,4, M. Leite de Morais1,2,4

1Faculté de Médecine Paris Descartes, Université Paris Descartes, Paris, France, 2Institut National de la Santé et de la Recherche Médicale, Unité 1151, Laboratory of Immunomodulation and Immunopathology, Institut Necker-Enfants Malades, Paris, France, 3Centre National de la Recherche Scientifique, UMR 8253, Laboratory of Immunomodulation and Immunopathology, Institut Necker-Enfants Malades, Paris, France.

Paradoxically, the Non-Obese Diabetic (NOD) mouse, a prototypic model of T-cell-mediated autoimmune diabetes, is highly prone to develop allergic reactions. Here, in a classical house-dust mite (HDM)-induced asthma model, we compared HDM and BALB/c mice responses and dissected the pattern of cytokine-producing cell subsets in situ. NOD mice exhibited exaggerated airway hyper-reactivity as assessed, using the FlexiVent device, by the resistance after methacholine challenge (101±9 vs. 4.0±0.8 cmH2O·ml⁻¹ at 25mg MCh, in NOD and BALB/c respectively, P=0.01). Allergic airway inflammation was enhanced, as reflected by increased eosinophil counts in the bronchoalveolar lavage fluids of NOD mice (5.6±0.6 vs. 0.7±0.2, P<0.01), as were circulating anti-HDM IgE levels (0.7±0.15 vs. 0.26±0.01, P<0.01). Interestingly, the pattern of cytokine-producing cells was clearly distinct in the lungs of asymptomatic NOD mice as compared to BALB/c. First, the proportions of IL-4, IL-13 and IL-5 producing CD4+ T cells were increased (13.2±3.1 vs. 6.7±1.9, 9.1±1.0 vs. 2.7±0.2, and 5.9±1.1 vs. 2.0±0.3 respectively, all P<0.01). Secondly, CD4+ T cells were the major IL-5 producers in NOD mice (60.8±4.0% TCRβ4+CD4+ among IL-5+ cells) while in BALB/c the source of IL-5 were type 2 ICS (60.6±6.7% TCRβ17+). Third, an exacerbated production of IL-17 was found in NOD mice which sources included conventional CD4+ T cells (3.1±0.3 vs. 1.1±0.2, P<0.01), CD8+ T cells (Il22+ T cell 11.2±1.6 vs. 1.6±0.1, P<0.01) and IL-17-producing Vγ9Vδ2+ T cells (2.8±0.4 vs. 0.9±0.04, P<0.05). Our results demonstrate that the NOD strain develops a unique form of severe allergic-induced asthma associated with exacerbated TH2 and IL-17-biased immune responses.
POSTER PRESENTATIONS

P.CS.03.12 Characterization of urticaria in children
P. H. Huang1,2, J. H. Lee1, L. L. Chang1
1Department of Pediatrics, Cardinal Tien Hospital, New Taipei City, Taiwan, 2Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan.

Introduction: The etiologies of acute urticaria vary, with the leading cause in infection in pediatric group. In contrast, infection only accounts for a minority of cause in the adult group. A previous study indicated that atopic diseases are related to acute urticaria, but not chronic urticaria. In addition, the percentage of acute versus chronic urticaria are different in children and adults. This study is to evaluate the age distribution of urticaria types, etiologies, and relationship with atopy in children.

Materials and Methods: Patients’ data are obtained retrospectively by chart review. Types of urticaria are classified according to 2018 revision of EAAC/GA2LEN/EDF/WAO Guidelines for Definition, Classification, Diagnosis and Management of Urticaria. Identification of etiologies are from notes and questionnaires to the family if necessary. The included patients are divided into 4 age groups (0-3, 3-6, 6-11, 12-18) to compare the differences among age groups.

Results: The results showed the ratio of acute versus chronic urticaria is 4:1. The overall age statistics revealed a male predominance and the female/male ratio is 0.76. Of all the identified etiologies, infection is the leading cause of acute urticaria (33.5%), whereas there is only 2.4% patients with chronic urticaria caused by infection. Allergic diseases were found in over one third patients with acute urticaria while only 11% in that with chronic urticaria.

Conclusion: Our findings suggested there was much difference in etiologies and relationship with atopy between acute and chronic urticaria in overall age groups. Further analysis addressing the different age groups will be further reported.

P.CS.03.13 The transcription factor EPAS1 links DOCK8 deficiency to atopic skin inflammation via IL-31 induction
K. Kunimura1, K. Yamamoto2, Y. Fukui3
1Kato Institute of Biomedical Research, Kyushu University, Fukuoka, Japan, 2Research Center for Advanced Immunology, Kyushu University, Fukuoka, Japan.

Introduction: Mutations of DOCK8 in humans cause a combined immunodeficiency characterized by atopic dermatitis. However, the molecular link between DOCK8 deficiency and atopic skin inflammation is unknown. Materials and Methods: The skin disease development was compared between Dock8−/− and Dock8+/− mice expressing TCR transgene. After stimulation with the cognate antigen, CD4+ T cells were used for cytokine production assay, microarray analysis and ChiP assay. IL-31 promoter activation, EMSA and immunoprecipitation were performed by standard techniques. Nuclear translocation of EPAS1 was examined in mouse embryonic fibroblasts generated from wild-type and Dock8−/− mice.

Results: We found that unlike Dock8−/− mice, Dock8+− mice spontaneously developed severe atopic skin inflammation, when crossed with transgenic mice expressing TCR with a particular antigen specificity. Upon stimulation, CD4+ T cells from Dock8−/− mice produced large amounts of IL-31, a major pruritogen associated with atopic dermatitis. This IL-31 induced by DOCK8−/− mice was dependent on the transcriptional induction in CD4+ T cells abrogated, and skin disease development in Dock8−/− mice. Although EPAS1 is known to form a complex with any hydrocarbon receptor nuclear translocator (ARNT) and control hyposon responses, EPAS1-mediated IL31 promoter activation was independent of ARNT, but in collaboration with SP1. In addition, we found that Dock8−/− acted as an adaptor and negatively regulated nuclear translocation of EPAS1. Conclusions: EPAS1 links DOCK8 deficiency to atopic skin inflammation via IL31 induction in CD4+ T cells.

P.CS.03.14 The role of CD133 in the development and immune modulation of SSEA-1+ lung stem/progenitor cells
C. Lia1, C. Chiu1, B. Chang1,2
1Graduate Institute of Immunology, College of Medicine, National Taiwan University, Taiwan, 2Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan.

Introduction: In the previous studies, we had identified a subset of lung stem/ progenitor cells from neonatal mice lungs, SSEA-1+ pulmonary stem/ progenitor cells (SSEA-1+PSCs). SSEA-1+PSCs are able to self-renew and differentiate into airway ciliated and alveolar cells. Interestingly, SSEA-1+PSCs but not SSEA-1− cells are capable to suppress allergic airway inflammation in OVA-induced allergic asthma mouse model. However, we found that SSEA-1+PSCs are further divided into two cell populations by CD133 expressing or not. In the present study, we analyzed the stem ness and functions of CD133+SSEA-1+PSCs and CD133+SSEA-1−PSCs. Materials and Methods: CD133+SSEA-1+PSCs and CD133+SSEA-1−PSCs were isolated from neonatal mice lungs. Cells were analyzed by qPCR, cell differentiation assays and 3D culture. OVA-induced allergic airway inflammation were used to analyze the immuno-regulatory function of the cell populations. Results: In comparison with the gene expression profile, CD133+SSEA-1+PSCs are expressed higher levels of airway-related genes than CD133+SSEA-1−PSCs. Although both the cell populations could differentiate into alveolar cells, only CD133+SSEA-1+PSCs are able to differentiate into airway ciliated cells. Conclusion: PSCs generated organoids during 3D culture, suggesting the stem ness of both cell populations. To determine the immuno-regulatory ability of CD133+SSEA-1+PSCs and CD133+SSEA-1−PSCs in allergic airway inflammation, cells are intravenously transferred into murine model of asthma. Results showed that the allergic airway inflammation are suppressed by both cell populations.

Conclusions: We demonstrated that neonatal mice lung-derived CD133+SSEA-1+ and CD133+SSEA-1− cell populations exhibited the stem cell activities and immuno-regulatory functions.

P.CS.03.15 Effect of chronic stress in a mouse model of contact hypersensitivity
A. Mackerness1, A. Sammersho1, M. Groettrup1,2
1University of Konstanz, Konstanz, Germany, 2Biotechnology Institute Thurgau, Kreuzlingen, Switzerland.

Chronic stress is known to have a suppressive effect on the immune system via the secretion of glucocorticoids mediated by the hypothalamus-pituitary-adrenal (HPA) axis. Interestingly, chronic stress is known to increase the susceptibility and disease progression of inflammatory skin diseases in humans. We studied inflammatory skin reactions in a well-established mouse model of contact dermatitis termed contact hypersensitivity (CHS) using the established contact allergens DNTB (2,4-dinitrothiocyanobenzene) and DNFB (2,4-dinitrofluorobenzene). DNFB-induced sensitization critically depended on the transcription factor EPAS1, and its conditional deletion in CD4+ T cells abrogated skin disease development in Dock8−/− mice. Although EPAS1 is known to form a complex with any hydrocarbon receptor nuclear translocator (ARNT) and control hyposon responses, EPAS1-mediated IL31 promoter activation was independent of ARNT, but in collaboration with SP1. In addition, we found that Dock8−/− acted as an adaptor and negatively regulated nuclear translocation of EPAS1. Conclusions: EPAS1 links DOCK8 deficiency to atopic skin inflammation via IL31 induction in CD4+ T cells.

P.CS.03.16 Immunological effects of omalizumab in a group of long-term treated asthma patients
L. Maggi1, B. Rossetti1, G. Montani1, A. Motacci1, A. Valtagggi1, A. Mazzoni1, E. Moggi1, F. Lisetta1, F. Annunziato1, L. Cosmi1
1University of Florence, Florence, Italy, 2Azienda Ospedaliero-Universitaria Careggi, Florence, Italy.

Background: The anti-IgE monoclonal antibody, omalizumab, has proven to be effective in the treatment of allergic severe uncontrolled asthma. The aim of the study was to evaluate the immunological effects of omalizumab, in a group of severe allergic asthma patients, treated since at least three years.

Methods: Immune cell subsets have been evaluated in a cohort of 15 allergic asthmatic patients treated with omalizumab, and the data have been compared with 12 allergic asthma patients treated with standard therapy.

Results: Omalizumab treated asthmatic patients showed lower frequencies of circulating plasmacytoid dendritic cells, and lower CD154 expression on CD4 T helper cells respect to the control group. Moreover, basophils and DCs from omalizumab treated patients had lower levels of IgE on their surface respect to the control group, and this was the consequence of the reduction of FcεRI expression, but also some FcεRI free of IgE were detected. In a longitudinal evaluation of two patients that started omalizumab treatment, the presence of FcεRI free of IgE was evident just after the first administration of the drug. Finally we performed in vitro experiments on basophils obtained from healthy donors, that show that omalizumab is able to detach IgE from their receptors.

Conclusions: Collectively these data indicate that long-term omalizumab treatment dampen type 2 inflammation acting on different cell types that play a pivotal role in the pathogenesis of allergic asthma, Moreover a possible novel activity of omalizumab, the ability to detach IgE from their receptors, is suggested.
POSTER PRESENTATIONS

P.C5.03.17
Targeting the IL-7R alpha as a potential therapeutic approach for allergic asthma

H. L. Mao1, E. Nguyen1, G. Bouchaud1, K. Henrio2, M. Cheminant1, S. Dehmann3, A. Magnan4, S. Brouard4
1Centre de Recherche en Transplantation et Immunologie UMR 1064, INSERM, Université de Nantes, Nantes, France, 2Institut de Transplantation Urologie Néphrologie, CHU Nantes, Nantes, France, 3INRA IBIA UR 1268, Nantes, France, 4INSERM UMR 1018, Institut du Thorax, Nantes, France.

Introduction: Asthma remains an important cause of morbidity and mortality. We herein provide for the first time a preclinical proof-of-concept for a novel therapeutic approach for allergic asthma using an anti-IL-7Ra mAb, which blocks both IL-7R and TSLP. Methods: We used a murine asthma model in which mice received 4 weekly percutaneous sensitizations and 2 weekly intranasal UUO challenges with total house dust mite (HDM) extracts as allergens. This model model corresponds to a mixed asthma phenotype in which the bronchoalveolar inflammation comprises both neutrophils and eosinophils. Asthmatic mice were then treated with an anti-IL-7Ra mAb or an isotype control every other day during the 2 weeks of IN challenges. Results: Anti-IL-7Ra mAb blocks STAT5 phosphorylation in mouse CD4+ T cells induced by either IL-7 or TSLP. Compared to control animals, anti-IL-7Ra-treated mice showed significantly lower airway resistance in response to methacholine as measured by flexiVent, associated with an improvement in lung histology. Anti-IL-7Ra treatment significantly decreased the mRNA expression of Th2 cytokines (IL-4, IL-5, and IL-13) and chemokines (CCL11 and CCL15) in bronchoalveolar lavage fluid (BALF) as measured by luminex, and decreased serum HDM-specific IgE as measured by ELISA. Leukocyte phenotyping by flow cytometry revealed a reduction of eosinophils, total lymphocytes, T cells, and especially IL2 in lung and BALF of anti-IL-7Ra-treated mice. Conclusion: Targeting the IL-7 Ra by a mAb, through its broad mechanisms of action, presents as a potential therapeutic approach for asthma.

P.C5.03.18
Whole-proteome profiling of primary human mast cells reveals evolutionary conservation of cell-type specific pathways

T. Plum1, T. Feyereisen2, M. Reiter3, J. Krijgsveeld4, H. Rodewald5
1Toulouse University, Dermatology and Allergology Department, Larrey Hospital, Toulouse, France, 2INSERM-Paul Sabatier Toulouse University, U1056 LIDEAR, Purpan Hospital, Toulouse, France, 3Toulouse University, Immunology Department, Rangueil hospital, Toulouse, France, 4Toulouse University, Ear Nose and Throat Department, Larrey Hospital, Toulouse, France, 5Toulouse University, Ophthalmology Department, Pierre Paul Riquet Hospital, Toulouse, France.

Introduction. Dupilumab is an antagonist of the Interleukin-4/13 receptors and was recently approved in Europe for use in adults with moderate-to-severe atopic dermatitis (AD). Our objective was to assess the impact of dupilumab therapy on both clinical (cutaneous, pulmonary, nasal and ocular symptoms) and biological (total and specific IgE, sIgE) parameters in real-life practice. Methods. Dupilumab was administered as labelled in the context of a Temporary Use Authorisation. Patients were evaluated initially (week 0, W0) and 16 weeks (W16) after the first injection. Follow-up parameters were: SCORAD (SCORAD), visual analogue scale (VAS) for sleep and pruritus, Dermatology Life Quality Index (DLQI), Asthma Control Test (ACT), fraction of exhaled nitric oxide (FeNO), expiratory flow-volume curves, ophthalmological and nasal symptoms, dosages of total and sIgE, and, using both single-testing and multiplex testing (LISA Diagnostic System and LISA Diagnostic System), IgE levels. Results. Nineteen patients were included (median age: 38 years). The median SCORAD was 49 at W0. SCORAD, DLQI, pruritus and sleep VAS decreased significantly at W16. There was no difference for the ACT or the flow-volume curves but a significant decrease was observed for the FeNO. Eight patients presented either a de novo conjunctivitis (n=3) or a worsening of their ophthalmological symptoms at W16. Four patients improved their rhinitis and one experienced worsening. Total IgE, sIgE against Malassezia or Staphyloccocus toxins, and ISAC sIgE (total of 517 values) decreased significantly at W16. However, there was no correlation with the SCORAD improvement. Conclusion. Dupilumab improves AD condition in real-life practice and modifies the sIgE sensitisation profile of the patients.

P.C5.03.19
Dupilumab in real life practice: patient's atopic profile, efficacy on atopic dermatitis and change in specific IgE

M. Tauber1, P. Apoil1, C. Richet2, G. De Bocanegra3, E. Mouchon3, M. Cassagne3, M. Marguery1, S. Hegay1, M. Konstantinou3, M. Severino4, C. Luthiargue1, F. Giordano-Labadie1, A. Didier1, C. Paul1
1Toulouse University, Dermatology and Allergology Department, Larrey Hospital, Toulouse, France, 2INSERM-Paul Sabatier Toulouse University, U1056 LIDEAR, Purpan Hospital, Toulouse, France, 3Toulouse University, Immunology Department, Rangueil hospital, Toulouse, France, 4Toulouse University, Ear Nose and Throat Department, Larrey Hospital, Toulouse, France.

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P.C5.03.20
Plasma cytokine profiles during peanut oral immunotherapy in severely peanut allergic adolescents

M. van der Heiden1, C. Carvalho-Queiroz2, C. Nilsson3, C. Paul2, A. Didier1, G. De Bonnecaze4, M. Tauber1
1Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, 2Sachs’ Children and Youth Hospital, Södersjukhuset, Stockholm, Sweden, 3Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Stockholm, Sweden.

Introduction. Peanut allergy is a major cause of anaphylaxis, hence effective treatment strategies for peanut allergy are warranted. Our previous work has shown that individualized omalizumab treatment (OT) allows a safe initiation and rapid up-dosing of peanut oral immunotherapy (pOIT) in severe peanut allergic adolescents. We aimed to longitudinally follow the cytokine profiles in plasma samples of severely peanut allergic adolescents (n=19) undergoing combined OT and pOIT and subsequently compare these profiles between treatment failures (TF) (n=6) and successes (TS) (n=13). Concentrations of plasma cytokines (IL2, IL4, IL5, IL10, IL12p70, IL13, IL21, GM-CSF, IFNg, TNFa) were determined by multiplex bead-based immunoassays and ELISA at 4 different time points: before starting OT (baseline), at the peanut challenge after OT prior to starting pOIT (challenge), at maintenance dose pOIT prior to OT reduction (stepdown), and at the final peanut protein challenge (final). Cytokine levels were relatively low and stable between the time-points. We noted an increase of IL-5 and IL-9 levels at the maintenance dose pOIT prior to OT reduction (stepdown) time-point in most individuals. Further, we observed higher concentrations of GM-CSF at the challenge time-point in the TF group. These data indicate that most individuals have an ongoing Th2/Th17-type of response during pOIT. The higher GM-CSF levels at the challenge time-point in the TF group suggest a more active basophil compartment in these individuals. Future research will focus on chemokine responses as well as B and T cells underlying the treatment.

P.C5.03.21
Human immunoglobulin: an effective treatment for severe atopic dermatitis

P. E. Walo Delgado1, P. Lapuente Suárez2, A. Rancancio Clavijo3, M. A. Ballester Martin3, A. De Andres Martin3
1Servicio de Inmunología, Madrid, Spain, 2Servicio de Dermatología, Madrid, Spain.

Introduction. Atopic dermatitis is the most prevalent inflammatory skin disease. Currently, there are a wide range of treatments that include phototherapy, topical treatment, cyclosporine and biological therapy with monoclonal antibodies. In severe cases, patients undergo prolonged therapy and high doses of drugs, which leads to the appearance of more adverse effects and comorbidities, such as increased risk of infections. Materials and method. We included 3 patients (24-year-old men, 58-year-old women, and 17-year-old women) with severe atopic dermatitis who had failed conventional treatment (including phototherapy, prednisone, cyclosporine, and Omalizumab). We have treated them with human immunoglobulin (IVIG), at doses of 0.8 mg / kg every 3-4 weeks, after signing informed consent and approval by the pharmacy committee of our hospital. Results All 3 patients had an important improvement of their symptom, with decreased pruritus and eczema. Two patients with asthma have presented improvement of respiratory symptoms. No adverse effects related to the treatment were reported. Conclusion. IVIG is an effective and safe alternative to treat severe atopic dermatitis due to its high capacity of immunomodulation. However, due to its high cost and limited production, it should be used in cases of refractory patients or when conventional treatment is contraindicated due to comorbidities.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
POSTER PRESENTATIONS

P.CS.03.22
Structural characterization of different profilin allergens towards IgE-epitope prediction
J. Wortmann, J. Schöpf, A. M. Reisenbichler, G. Hofes, W. Keller; Institute of Molecular Biosciences, University of Graz, Graz, Austria.

Members of the profilin family are important panallergens causing respiratory allergy and oral allergy syndrome. Because of the high structural conservation within this family, profilin allergens serve as important targets for IgE-binding and epitope prediction studies. The aim of this project is to elucidate the structure and biophysical characterization of five profilin allergens. The folded, recombinantly produced proteins will also be used for experimental determination of cross-reactivity between important respiratory as well as food allergens. The profilin family members included in this research project are the food allergens Cuc m 2 from melon (Cucumis melo) and CIt s 2 from orange (Citrus sinensis), the pollen allergens from olive tree Ole e 2 (Olea europaea), and timothy grass Phl p 12 (Phleum pratense) as well as the allergen Tyr p 36 from the storage mite Tyrophagus putrescentiae.

So far, all five profilin allergens could be recombinantly expressed and purified. Biophysical characterization including Circular Dichroism, Differential Scanning Fluorimetry and Size Exclusion Chromatography revealed the structural integrity of the allergens. Obtaining immunological data regarding cross-reactivity will enable more accurate IgE-epitope predictions using a structure based IgE-epitope mapping approach (Dai’Antonia et al.).

P.CS.04.04
Allergy, asthma and therapy - Part 4

P.CS.04.01
Activated intestinal epithelial cells conditioned with 2'-Fucosyllactose and Cpg-ODN might instruct moDC to drive Th1 differentiation
V. Ayechu-Muruzabal, A. Kostadinova, S. Overbeek, B. Stahl, J. Garsen, B. van ’t Land, L. Willemsen; 1,2University of Amsterdam, Amsterdam, The Netherlands; 3Interuniversity Messerli Institute/Institute of Pathophysiology and Allergy Research, Interfaculty of Pathophysiology, Infectology and Immunology, Medical University of Vienna, Vienna, Austria.

The interuniversity Messerli Research Institute, Vienna, Austria, is an interfaculty research institute that gathers expertise from the Medical University of Vienna, Vienna, Austria, St. John’s Institute of Dermatology, School of Basic & Medical Biosciences, King’s College London & NIHR Biomedical Research Centre at Guy’s and St. Thomas’ Hospital and King’s College London, London, United Kingdom, Breast Cancer Now Research Unit, School of Cancer & Pharmaceutical Sciences, King’s College London, London, United Kingdom.

The creation of tailor-made antibodies is interesting both in the clinics and in research. PIPE (Polymerase Incomplete Primer Extension) cloning is a cutting-edge method that allows fast assembly of antibody constructs. It furthermore enables the creation of antibodies of several classes sharing the same binding region (Ilieva et al., 2017). We here present PIPE-cloned IgG, IgE, and IgG targeting the major birch pollen (Betula verrucosa) allergen Bet v 1. PIPE cloning was used to create vectors of several antibody classes (IgG, IgE, IgG), sharing the same variable region against Bet v 1 (Levin et al., 2014). Plasmids were expressed in Exp293F cells and purified with affinity chromatography. Concentration was measured via BCA protein assay and purity and correct assembly was confirmed by SDS PAGE, the specificity of all classes of recombinant antibodies against Bet v 1 was tested in a dot blot, of IgG additionally in an ISAC112 microarray. Affinity chromatography resulted in specific isolation of correctly assembled antibodies, as was confirmed via SDS PAGE. Overall yields were in the range of several hundred micrograms (IgE) to milligrams (IgG4). All of the produced antibodies specifically bound to Bet v 1 in a dot blot, IgE recognised Bet v 1 in an ISAC112 allergen microarray with high specificity. PIPE cloning is a time-efficient method to obtain a PIPEline for targeting Bet v 1 for studying class-specific antibody function in type 1 allergy. The work was supported by the Austrian Science Fund FWF grants MCCa W1248-B30 and F4606-B28 to EJJ.

P.CS.04.04
Antibody PIPEline by PIPE cloning: Efficient production of Bet-v-1-specific antibodies of different classes sharing the same variable region
V. K. Köhler1, J. F. Singer1, K. M. Ilieva1, L. C. Pranger2, S. N. Karagiannis3, E. Jensen-Jarolim2,3; 1Interuniversity Messerli Institute/Institute of Pathophysiology and Allergy Research, Vienna, Austria, 2Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectology and Immunology, Medical University of Vienna, Vienna, Austria, 3St. John’s Institute of Dermatology, School of Basic & Medical Biosciences, King’s College London & NIHR Biomedical Research Centre at Guy’s and St. Thomas’ Hospital and King’s College London, London, United Kingdom, 4Breast Cancer Now Research Unit, School of Cancer & Pharmaceutical Sciences, King’s College London, London, United Kingdom.

The development of tailor-made antibodies is of great interest both in the clinics and in research. PIPE (Polymerase Incomplete Primer Extension) cloning is a cutting-edge method that allows fast assembly of antibody constructs. It furthermore enables the creation of antibodies of several classes sharing the same binding region (Ilieva et al., 2017). We here present PIPE-cloned IgG, IgE, and IgG targeting the major birch pollen (Betula verrucosa) allergen Bet v 1. PIPE cloning was used to create vectors of several antibody classes (IgG, IgE, IgG4), sharing the same variable region against Bet v 1 (Levin et al., 2014). Plasmids were expressed in Exp293F cells and purified with affinity chromatography. Concentration was measured via BCA protein assay and purity and correct assembly was confirmed by SDS PAGE, the specificity of all classes of recombinant antibodies against Bet v 1 was tested in a dot blot, of IgG additionally in an ISAC112 microarray. Affinity chromatography resulted in specific isolation of correctly assembled antibodies, as was confirmed via SDS PAGE. Overall yields were in the range of several hundred micrograms (IgE) to milligrams (IgG4). All of the produced antibodies specifically bound to Bet v 1 in a dot blot, IgE recognised Bet v 1 in an ISAC112 allergen microarray with high specificity. PIPE cloning is a time-efficient method to obtain a PIPEline for targeting Bet v 1 for studying class-specific antibody function in type 1 allergy. The work was supported by the Austrian Science Fund FWF grants MCCa W1248-B30 and F4606-B28 to EJJ.

P.CS.04.05
Antibody PIPEline by PIPE cloning: Fast production of human monoclonal IgG1, IgG4 and IgE antibodies specific for beta-lactoglobulin from milk
C. L. Pranger1, J. F. Singer1, K. M. Ilieva1, S. N. Karagiannis3, E. Jensen-Jarolim2,3; 1Interuniversity Messerli Institute/Institute of Pathophysiology and Allergy Research, Interfaculty of Pathophysiology, Infectology and Immunology, Medical University of Vienna, Vienna, Austria, 2St. John’s Institute of Dermatology, School of Basic & Medical Biosciences, King’s College London & NIHR Biomedical Research Centre at Guy’s and St. Thomas’ Hospital and King’s College London, London, United Kingdom, 4Breast Cancer Now Research Unit, School of Cancer & Pharmaceutical Sciences, King’s College London, London, United Kingdom.

The need for large and high quality antibody production is evident in many areas of research. The newest approach for the production of monoclonal antibodies (Ilieva et al., 2017). Here we use this method to generate antibodies of diverse subclasses (IgG, IgE, and IgG4) against the major milk allergen beta-lactoglobulin, termed Bos s 5. Methods: Vectors of antibodies with the variable region against Bos s 5 (Jylhä et al. 2016) were assembled using the PIPE cloning method and transformed into E. coli. Clones were validated by colony-PCR and afterwards expressed in the Exp293F cells. After affinity chromatography, antibody yields were measured with MCA protein assay and recombinant antibodies were controlled by SDS-PAGE for correct assembly and checked for specificity to BLP. The recombinant clone yield was 2.2 mg of IgE, 0.6 mg of IgG1 and 1.9 mg of IgG4 antibodies. All of the antibodies were able to bind to BLP. Therefore, the method is a very useful tool for antibody production and can be used for the production of antibodies with a specific binding to BLP. The work was supported by Austrian Science Fund FWF, grants MCCa W1248-B30 and SB F4606-B28.
POSTER PRESENTATIONS

P.C5.04.06
Title: T cell responses to sublingual treatment with recombinant Mal d 1
C. Kitazumi, B. Nagi, T. Kinecyan, B. Bohl
Medical University Vienna, Vienna, Austria.

Background: More than 70% of birch pollen-allergic individuals develop birch pollen-related food allergy (BPRFA), most frequently to apple. We recently conducted a clinical study of sublingual immunotherapy (SLIT) with recombinant birch allergen, (r)Bet v 1, apple allergen, rMal d 1, or placebo for 16 weeks. Interestingly, only the patients receiving rMal d 1 significantly improved BPRFA. In the current study, we analysed changes in the T cell compartment of the rMal d-treated patients over the course of treatment. Methods: We investigated T cell reactivity and the expression levels of the key cytokines IL-4, IL-5, IL-13, IFN-γ and TGIF in response to specific stimulation by thymidine incorporation and RT-qPCR, respectively. Additionally, we analysed changes in the relative numbers of CD4+ memory T cell subsets, namely Th1, Th2, Treg and Th17, with subset-specific surface markers and flow cytometry. Results: The proliferative response to rMal d 1 was reduced over the course of treatment, however, this was not accompanied by changes in the expression levels of cytokines. Almost all of the CD4+ memory T cell subsets analysed were unchanged with the notable exception of pro-allergic Th2 cells (CD27-, CORT2+, CC4+), which were significantly decreased already after 4 weeks of treatment. Conclusion: SLIT with recombinant apple allergen shows promising results, however, despite clinical improvements, only minor changes in the T cell compartment were observed. Supported by: OnNB project 16620, the Austrian Science Fund [projects K106 and SFBF4610], Biomay AG, and the Christian Doppler Research Association, Vienna, Austria.

P.C5.04.07
EFFECT OF AIR POLLUTION ON PATIENTS WITH BRONCHIAL ASTHMA IN GEORGIA
R. Sepiashwall*, M. Chikhladze*, S. Gamrekelidze*

‘Pepsoc Friendship University of Russia, Moscow, Russian Federation, ‘National Institute of Allergology, Asthma and Immunology of Georgian Academy of Sciences, Tskhaltubo, Georgia

The study aimed to establish the correlation between the concentration of phadiatop, total IgE levels in the blood in patients with bronchial asthma and the concentration of specific air pollutants in terms of annual calendar of flowering plants in West Georgia. In the study were involved 45 patients (24 males and 21 females) of different ages with bronchial asthma. The study included: I step - allergodiagnostic using “Immuno-CAP 100”, II step - monitoring of aeropolutants concentration by using aeropolinometer “Burkard Trap”. Patients had high titers of total IgE, which amounted to an average of 273 (N 33-90), while the average concentration of phadiatop was 96 (N 710). Patients with bronchial asthma of a specific positivity of specific IgE to the weeds (Wx2) - ambrosia, plantain, clasp/tarragon, atriplex - in 25 (55%) on average; tree dust (Tx9) - alder, lactarius piperatus, nuts, oak, willow - 16 (35%); and cereals (Ga1) - festuca pratensis, loliun temulentum, timo1 grass, pea - 8 (17%); Mx2 - Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, Candida albicans, Alternaria alternate - 11 (24%) was revealed, only in 1 (2%) case we cannot established the allergy specific IgE. From January to April 2017, there were revealed a high concentration of allergens, by high allergenization and widespread; especially high concentrations were found in birch, alder and common busy, while from aeropolutants of low allergenization poplar, elm, willow and plane tree were detected. High degree correlation between above-mentioned markers proves its clinical importance/Value with respect to bronchial asthma. This publication was prepared with the support of the “RUDN University Program 5-100”.

P.C5.04.08
In vivo T regulatory cell regulation during human rhinovirus infection

1) Imperial College London, London, United Kingdom, 2) Medical University Vienna Department of Oto-Rhino-Laryngology, 3) Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectology and Immunology, Medical University of Vienna, Vienna, Austria.

Rationale: Respiratory infections with human rhinoviruses (HRV) are strongly associated with asthma exacerbations and pose a severe health risk for allergic individuals. How HRV infections and chronic allergic diseases are linked, and which role HRV plays in the breaking of allergen-specific tolerance is unknown. T regulatory cells (Tregs) play an important role in the induction and maintenance of immune tolerance. Therefore, the aim of this study is to investigate the effects of HRV on Tregs during asthma exacerbations.

Methods: Healthy and atopic individuals were experimentally infected with HRV16 in vivo. Peripheral blood mononuclear cells (PBMCs) were obtained before infection and three days after infection. Tregs were sorted from the PBMCs according to their flow cytometric profile CD4+CD25+CD127- and were analyzed with NGS.

Results: We have found that upon viral infection in both asthmatics and healthy individuals an antiviral response is induced in Tregs, including upregulation of MX1, STAT1, IFI44L, IRF7/9, OAS3. In healthy individuals CCL5 was downregulated, while unchanged in asthmatics.

Conclusions: Tregs from healthy and asthmatic individuals both show an anti-viral response after RV infection. However there are also clear differences in response between Tregs from healthy and asthmatic individuals. These differences in response might affect Treg functions, level of inflammation, chronicity and viral clearance. Together this data suggest that Treg functions might be altered or impaired during HRV infections, which may contribute to asthma exacerbations.

P.C5.04.09
Qualitative and quantitative comparison of pollen allergen exposure between horses and humans. A collaborative study with the Austrian Pollen Information Service
A. L. Koratree, K. Basti1, G. Hofstetter, K. Kufner, M. Krentel, U. E. Berger, E. Jensen-Jarolim*1

1) The Interuniversity Messerli Research Institute, University of Veterinary Medicine Vienna, Medica, Vienna, Austria; 2) Medical University of Vienna Department of Oto-Rhino-Laryngology and Allergy Information Research Unit, Vienna, Austria; 3) Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectology and Immunology, Medical University of Vienna, Vienna, Austria.

Background: In our recent study we revealed that IgE patterns in IAC allergen microarray (Einhorn et al, Allergy 2018) differed between horses and humans, suggesting different allergen exposure.

Aims: We therefore aimed to collect pollen and plant samples from horse paddocks and meadows and compare to the pollen counts assessed for humans by the Austrian pollen Information Service.

Methods: Pollen were collected, on paddocks and pastures in four different horse stables, using pollen traps in April and in June 2018. The surrounding vegetation was examined botanically to correlate the pollen with the occurrence of allergic plants.

Results: The overall pollen count was higher in early spring. In spring mostly pollen from Picea, Quercus and Fagus were found and in summer Poaceae, Urticaceae, Sambucus and Plantago pollen. The same pollen species as relevant for humans occurs in the equine environment, however, largely differing in terms of quantitative composition.

Conclusions: This study will provide evidence whether the human APS (Austrian Pollen Information Service) can be useful for owners of sensitized horses.

This study is supported by Austrian Science Fund FWF grant SFB F4606-B28 to Ei.

P.C5.04.10
Qualitative multiplex detection of allergen-specific IgG and IgA on a microarray
G. Feyzkhanoova*, O. Smolodovskaya1, S. Velashin1, M. Filipov1a, E. Antonova1, L. Pavlushkina1, T. Filatova1, A. Rubina1

1) Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation, 2) Medical University of Vienna Department of Oto-Rhino-Laryngology and Allergy Information Research Unit, Vienna, Austria

Allergen specific IgG (sIgG) is the main marker of the hypersensitivity type I in atopic individuals. However, in certain cases adverse reactions or their absence cannot be explained only via IgE diagnostics. Detection of allergen-specific immunoglobulins of other classes, such as specific IgG (sIgG) or specific IgA (sIgA), and specification of their role in allergic disorders can give additional information which might improve allergy diagnostics.

For that purpose a microarray for multiplex qualitative determination of sIgG and sIgA to 31 allergens was developed. Microarray includes semispherical gel pads containing allergen extracts or individual proteins belonging to different groups: pollen, indoor or food allergens. 10 µl of the blood serum is necessary for the analysis. After the incubation of microarray with diluted blood serum (1:15) microarray is developed with anti-human antibodies conjugated with fluorescent dyes. sIgG and sIgA profiles were obtained on the microarrays for 30 patients with different allergic diseases, using as developing antibodies anti-IgG-Cy3, anti-IgA-Cy5 or the mix of these antibodies. The signals for individual antibodies and for their mixture agreed within standard deviation of the analysis, which indicates that the developing system was selected properly. Also, the obtained patterns of sIgG and sIgA responses didn’t coincide with the pattern of sIgE response defined previously.

The assay procedure developed is simple and may be used for screening both in adults and children to specify the relation between immunoglobulins G and A against allergens of different origin.

This work was supported by the Russian Science Foundation, Grant No. 14-50-00060.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Authors of Sensitized Horses.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

408

POSTER PRESENTATIONS

P.CS.04.11
Macrophage exposure to antibiotics increases asthma severity in offspring mice

L. Linge1, J. Gray2, H. Dechmikh3, P. Koenig4, I. Lewiowicz5
1Institute of Anatomy, Luebeck, Germany, 2Division of Neonatology, Perinatal and Pulmonary Biology, Cincinnati Children’s Medical Center, Cincinnati, United States, 3Division of Immunobiology, Cincinnati Children’s Medical Center, United States.

Within the last 40 years, asthma prevalence has tripled. As genetics do not change this rapidly, it is thought that recent changes in life-style in developed nations is increasing the risk of developing allergic asthma in genetically susceptible individuals. Recent epidemiological studies link early life exposure to antibiotics or other microbiota-changing events with increased risk of the child to develop asthma. In this study, we seek to understand the role of early life microbiota disruption on asthma pathogenesis in mice. Offspring of mothers exposed to antibiotics (ABX) in drinking water (1mg/mL of Ampicillin, Gentamicin and Vancomycin) from embryonic day 15 (E15) to postnatal day 14 (P14) displayed a markedly exacerbated allergen-induced asthma phenotype with high airway hyperresponsiveness (AHR), increased numbers of IL-17A+ innate lymphoid cells (ILC), neutrophils, and high levels of IL-22 in the bronchial lavage. A more elaborate ABX exposure regimen (day E15 to P15) was associated with less severe exacerbation of AHR, recruitment of ILC, but had limited impact on airway neutrophil and BAL cytokine levels. Interestingly, the limited ABX exposure regimen was also associated with aleuvalis enlargement and increased airway permeability. These observations suggest that early microbial exposures in critical windows after birth limit pathogenic immune responses in a sequential manner, and suggest that proper microbial colonization might have a previously unrecognized role in promoting normal lung maturation. Disrupting such colonizations may cause inadequate immune responses later in life.

P.CS.04.12
Immunoglobulin heavy-chain repertoire profiling of memory B cell, plasmablasts and plasma cells from peripheral blood of individuals with birch pollen allergy

A. I. Mikelovic1, M. A. Turchaninova2, E. A. Komche3, E. S. Egorov4, D. B. Staroverov5, Y. B. Lebedev6, D. M. Chadakov7, 1-3, 2, 4

The chronic wounds are stopped in the inflammatory phase, thus permitting the regeneration of the same. Silver-like nanoparticles have antibacterial properties, these have been used in a wide range of biomedical applications due to their selective toxicity in microorganisms and low immunogenicity. In addition, the use of immunomodulators contributes to inflammatory regulation to promote healing and regeneration.

Formulate a biosynthetic dressing loaded with silver nanoparticles and a TNFα inhibitor that promotes tissue regeneration and bacterial inhibition in an experimental model of chronic wounds.

Methodology. Chitosan gel was formulated. Silver nanoparticles were synthesized and their size was corroborated by SEM, TEM and IR. Dissolution time tests of the pharmaceutical form were carried out according to the Mexican pharmacopia. A bacterial inhibition test was performed in solid culture media type antibiogram. An experimental model of chronic wounds was established. Tissue histologies were performed. The genes of TNFa, TGFβ1, MMP8, IL-1 and IL-17 were measured.

Results. Chitosan with 90% purity was obtained. Subsequently, the chitosan was solubilized until it became semi-solid. Silver nanoparticles of 30 nm were synthesized by chemical synthesis. A formula was standardized with previous compounds and a TNFα inhibitor. In the solubility tests, formula was kept releasing drug for 5 days at different pH. Bacterial inhibition was achieved by visualizing agar bacteria inhibition. The inflammation was decreased histologically. As well as a decrease in the expression of proinflammatory cytokines was observed in the treated group. Healing of the wound was achieved before 15 days compared to the negative control.

P.CC.04.02
HIV-Induces IL-18 from intestinal epithelial cells that causes cell death, increased intestinal permeability & microbial translocation

A. Ahmad, O. Allam, S. Samaranai

CHU Ste-Justine/University of Montreal, Montreal, Canada.

IL-18 is a pro-inflammatory cytokine belonging to the IL-1 family. It has been shown that HIV infection is accompanied by an imbalance in the production of IL-18 and of its natural antagonist, the IL-18 Binding Protein (IL-18BP). The infection is also accompanied by intestinal inflammation, increased intestinal permeability and microbial translocation. However, little is known concerning the potential role of the cytokine in the causation of the intestinal pathology. Here we show that incubation of HIV with human intestinal epithelial cells (IEC) increases production of IL-18 and decreases that of IL-18BP from the IEC. The cytokine induces apoptosis in IEC in a time- and dose-dependent manner via activating caspase-1 and -9. It modulates the expression of several Tight and Adherens Junction proteins such as occludin, claudin 2 and β-catenin. It also disorganizes F-actin expression in the IEC. The cytokine decreases transepithelial electrical resistance (TEER) and increases permeability in the IEC monolayers. It also increases the expression of phosphorylated myosin II regulatory light chain (p-MLC) and myosin light-chain kinase (MLCK), and decreases activation of STAT-5 in the IEC. A Rho-kinase (ROCK)-specific inhibitor suppresses the cytokine-induced increase in p-MLC. Interestingly, the levels of the cytokine correlate with those of LPS in the circulation in HAART-naive and HAART-treated HIV-infected individuals, Elite controls as well as in healthy controls. Taken together, our results suggest that the increased concentrations of IL-18 play a role in increased intestinal permeability and microbial translocation observed in HIV-infected individuals. Thus targeting the cytokine may ameliorate intestinal pathology in HIV-infected individuals.

P.CC.04.03
Higher frequencies of lymphocytes expressing the natural killer group 2D receptor and cytotoxic potential of NK cells in patients with Behcet disease

M. Bonacini, A. Soriano, Z. Alessandro, E. Calo, L. Cinino, F. Muratore, L. Fontana, M. Parmeggiani, C.Salvarini, S. Caci

Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy.

This study aimed to identify a specific profile of circulating Natural Killer (NK), NK-T and T cells able to discriminate patients with Behcet disease (BD) and Healthy controls (HC). Method: Peripheral blood mononuclear cells (PBMCs) were collected from 38 BD patients and 15 HC. The frequencies of NK, NK-T and T cells expressing CD16, CD69, NKGD2, NKp30, NKp46 and NKp44 were assessed by flow cytometry. Cytotoxic potential of NK cells was evaluated as being the percentage of cells expressing the degranulation marker CD107a after incubation with K562 cells. The levels of 27 cytokines were determined in plasma with a multiplex bead-based assay. Results: Higher percentages of NK, NK-T and T cells expressing NKGD2 were detected in PBMCs of BD patients than HC. ROC curve analysis showed that the evaluation of NKGD2+ NK, NK-T and T cell percentages discriminated between BD patients and HC. Moreover, there was a positive correlation between the BD Current Activity Form (BDCAF) scores and the frequencies of NKGD2+ NK and NK-T cells. A higher frequency of NK cells expressing CD107a was induced in PBMC from BD patients when compared to incubation with K562 cells. CD107a+ NK cells after the degranulation assay could help the clinicians in BD patients management. The increased expression of NKGD2 in BD patients is likely involved in disease pathogenesis.
POSTER PRESENTATIONS

P.C6.01.04
Evolutionary conserved cell- and immunobiological processes during tissue regeneration: comparative studies in an invertebrate model
K. Bodeć, E. Rumpler1, B. Kokhonyuk, P. Németh1, P. Engelmann1
1Department of Immunology and Biotechnology, Clinical Center, Medical School, University of Pécs, Pécs, Hungary, 2Department of Comparative Anatomy and Developmental Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary.

It is well known that annelid (segmented) worms possess strong regeneration capacity. However, limited information is available about the interactions of regeneration and immune-related mechanisms in earthworms. Our aim was to compare cell proliferation vs. cell death and the expression of certain immune-related genes in the course of anterior/posterior regeneration of the earthworm Eisenia andrei. To enumerate cell proliferation Click-iT EdU assay was applied following 2 and 4 weeks of regeneration. It was combined with FITC-coupled anti-EFCC mAbs to assess the involvement of different immuneocytes (e.g. coelomocyte) subsets in the regeneration process. Apoptotic activity was detected by TUNEL assay. Q-PCR analysis was executed to evaluate the expression of mRNA targets in the regenerating segments of E. andrei. A high number of proliferating cells were detected after 2 weeks that is decreased by the 4 weeks only in the posterior blastema. In contrast, the apoptotic activity was observed throughout the regeneration. Immunostaining revealed that coelomocyte subsets were accumulated in the blastema mainly during the posterior regeneration. Pattern recognition receptor genes evidenced a decreased pattern (except scavenger receptor) compared to intact animals. Several genes have similar expression pattern (TLR, LBP) during anterior/posterior regeneration, except the antimicrobial molecules (lysozyme). Evaluation of cellular biological events and immune-related gene expression revealed characteristic differences during anterior/posterior regeneration that is a novel observation in the field of invertebrate (earthworm) immunity. This work was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and Medical Research Foundation, University of Pécs.

P.C6.01.05
Soluble triggering receptor expressed on myelocytes -1 is strongly correlated with disease activity in systemic lupus erythematosus
I. Gioukourelas, A. Gkantaras, A. Georgiadou, P. Boura
Clinical Immunology Unit, 2nd Internal Medicine Department, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Background: Soluble Triggering Receptor Expressed on Myelocytes -1 (sTREM-1) is an innate immunity receptor, which participates in inflammatory reactions. Its serum levels reflect the magnitude of systemic inflammatory response and can discriminate between infectious and non-infectious causes. Its role in systemic lupus erythematosus (SLE) is unknown. In this study, we examined sTREM-1 in SLE patients with regard to disease activity.

Methods and Patients: Sixteen patients with SLE were enrolled. Diagnosis was based on the 1982 revised American College of Rheumatology (ACR) criteria. Disease activity was measured with the SLE Disease Activity Index 2000 (SLEDAI-2K). sTREM-1 levels were determined by Enzyme Linked Immunosorbent Assay (ELISA) in serum samples. Seventeen age- and sex-matched healthy individuals comprised the control group. Statistical analysis was performed with the SPSS package; p<0.05 was considered significant.

Results: Serum sTREM-1 was significantly higher in SLE patients than in normal controls. Its levels were strongly correlated to SLEDAI-2K. Conclusions: Serum sTREM-1 is strongly correlated to disease activity in SLE, probably reflecting the generalized activation of the innate immunity response in this disease and may be an additional biomarker in SLE.

P.C6.01.06
Investigating the innate training potential of the vaccine adjuvant alum
A. L. Gorman, E. C. Lovelle
Trinity Biomedical Sciences Institute, Dublin, Ireland.

Vertebrate immunity is classically divided into innate and adaptive immune responses. Recent findings have challenged the classical view of innate versus adaptive immunity, suggesting that innate cells can retain some "memory" of past immunological insults. This "trained immunity" which allows for primed cellular responses to secondary infections is independent of T and B cells and is mediated by innate cells such as monocytes/macrophages and NK cells. While it has been shown that certain live vaccines such as BCG can induce trained immunity, the effects of particulates such as the widely used vaccine adjuvant alum have not been addressed. Alum has been predominantly used in many vaccines due to its ability to enhance immunopotentiating and safety profile. However, how this widely used adjuvant elicits its effects remains elusive. We have shown that alum-trained macrophages adopt a distinct morphology in addition to an altered capacity for cytokine secretion upon re-stimulation with lipopolysaccharide in vitro. Using a NanoString inflammation panel to characterize the transcriptional profile of alum-trained cells we have found that training by this adjuvant downregulates proinflammatory factors. Further investigation into the in vitro effects of alum-trained macrophages will help shed light on the mechanism by which this particulate exerts its innate training effects in macrophages.

P.C6.01.07
Complement C3 and C4 are useful markers for predicting Bipolar disease severity and monitoring patients under psychotropic treatment
H. Hachicha1, N. Housmat1, R. Feih1, S. Fekri1, F. Ayadi1, A. Maouad1, A. Ayadi1, S. B. Hamadou1, J. Aloulou1, H. Masmoudi1
1Immunology Department, CHU Habib Bourguiba, Sfax, Tunisia, 2Psychiatric department, CHU Béni Chaker, Sfax, Tunisia.

Abnormalities of the immune system have recently been shown to be implicated in bipolar disease (BD). BD patients have been also reported to exhibit increased proinflammatory cytokine levels indicating the role of inflammation in this disease. In our study we aimed to find out whether complement system (C3 and C4 fractions) and immunoglobulins (Ig) are abnormal in BD and to explore the effect of psychiatric treatment on these psychotropic variables. This study was conducted during 36 months on 90 subjects (45 patients with manic relapse - group 1, 45 patients with depressive relapse - group 2) having no history of autoimmune disease and 45 age and sex matched controls. Mean plasma IgG and complement C3 levels were significantly higher in bipolar patients with manic relapse (r = 0.034, p = 0.040). The plasma level of the C3 and C4 fraction was correlated with EGF score (r = 0.375, p = 0.016, r = 0.340, p = 0.016). After treatment, there was a statistically significant increase in mean plasma IgG and IgA levels (p = 0.006, p = 0.009) and a decrease in the mean plasma C4 complement level (p = 0.004). Mean plasma IgM levels were significantly lower on sodium valproate treated patients (p = 0.004). Under atypical antipsychotics, the mean plasma level of the C3 fraction was statistically lower (p = 0.028) whereas under conventional antipsychotics it was statistically higher (p = 0.048). Among the variables studied, the complement system seems to be closely related to manic relapse, its severity, and psychotropic treatment.

P.C6.01.08
Assessment of autophagy function in systemic lupus erythematosus in respect of hyperlipidemia and immunosuppressive drugs
A. S. Hamadda1, m. i. aref; a. m. rabe2
1Al Azhar university hospital, Cairo, Egypt, 2Aswan university hospital, Aswan, Egypt.

Background: Autophagy is an orchestrated homeostatic process to eliminate unwanted proteins and damaged organelles in addition to regulation of lipids.

Objective: Assessment of autophagy focusing on lipids regulation in patients with SLE.

Patients and Methods: Subjects were divided into three groups. Group 1 included 60 newly diagnosed SLE patients before receiving any treatment, group 2 included the same subjects after 1 group of subjects after 3 months of immunosuppressive drugs and group 3 included 30 matched healthy donors as a control group. Disease activity was assessed by (SLEDAI) score, lip profile was measured in addition to evaluation of lipids uptake, enhanced phagocytosis and intracellular killing ability of monocytes and neutrophils using Sudan Black B & Nitroblue tetrazolium stains.

Results: There was a positive correlation between total cholesterol, LDL and triglycerides and disease activity(SLEDAI score) (r = 0.677, r = 0.603 and r = 0.718; respectively). On the contrary, there was a negative correlation between HDL and disease activity(r = -0.396). Furthermore, there was a negative correlation between lipid content of cells and intracellular killing disease activity(r = -0.258 and r = -0.324; respectively). After 3 months, comparing group 2 to group 1, there was a significant increase in cholesterol, LDL and triglycerides(P=0.027, P=0.021 and P=0.017; respectively) while HDL showed insignificant difference(P=0.0740). Lipid content in cells and intracellular killing significantly decreased(P=0.0322 and P=0.0271; respectively).

Conclusion: Autophagy is deficient in patients with SLE so they are more susceptible to infections and dyslipidemia, aggravated by immunosuppressive drugs. Consequently, lipid lowering drugs are definitely required to decrease comorbidity.

P.C6.01.10
The "Who is Who" of dermal infiltration in fibrosis developing fos-related antigen-2 mice
J. Haub1, V. K. Rakoci1, L. Lorenz1, J. Steinbach2, E. Wagner3, D. Schuppan2, K. Steinbrink1, 4
1Department of Dermatology, Mainz, Germany, 2Research Center for Immunotherapy, University Medical Center Mainz, Germany, 3Institute of Translational Immunology, Mainz, Germany, 4National Cancer Research Center, Madrid, Spain.

Systemic sclerosis is a complex and incompletely understood disease, resulting in skin and organ fibrosis. Cutaneous fibrosis is characterized by disproportionate accumulation of collagen and other extracellular matrix substances. However, involvement of innate immune cells and the sequence of inflammatory events in the early inflammatory phase of the disease have not been addressed so far.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

Page 409
Therefore we studied the murine model of systemic sclerosis: The fos-related antigen-2 mouse (fra-2). Fra-2 mice overexpressing fra-2 under MHCI promoter control and spontaneously developing vasculopathy and fibrosis in the skin and intestinal organs. In Fra-2 mice the inflammatory response peaks at week 7-9 of life. By week 12 mice develop massive dermal and pulmonary fibrosis leading to dyspnea by week 17.

At week 12 histological analysis of Fra-2 mice showed a significant enhancement of dermal thickness and a pronounced cutaneous collagen accumulation (H&E, Goldner’s trichrome, hydroxyproline assay).

Further immunohistochemical analysis of the blood and skin demonstrated an early cellular infiltrate consisting of activated myeloid cells (Ly6C+MHCIi). This myeloid infiltration peaked at week 9 and comprised CD11b+CD310b+ as well as CD11b+CD206+ cells, indicating an infiltration of terminally differentiated macrophages in sclerotic-prone skin. Along with myeloid cells Fra-2 mice exhibit pronounced levels of activated T-cells (CD4+CD25+), but a reduced proportion of CD4+Foxp3+ regulatory T-cells in the skin, lymph nodes, lung.

Our findings suggest that reduced Treg frequencies accompanied by a massive myeloid and lymphoid infiltration contribute to fibrosis development in Fra-2 mice.

This study is financially supported by the German Research Foundation (Collaborative Research Center CRC/7156).

P.C6.01.11
284 RECEPTOR REGULATE COLITIS DEVELOPMENT VIA TISSUE REPAIR MECHANISM.

T. Kim, J. Kim, B. Park, G. Park, S. Lim, K. Lee;
Korea University College of Medicine, Seoul, Korea, Republic of.

Ulcerative colitis is a chronic relapsing form of inflammatory bowel disease (IBD) that causes inflammation and ulcers in the colon. Inflammatory responses are important in the initiation and progression of inflammatory bowel disease (IBD). 284 (CD244), a member of SLAM family play a crucial role in inflammatory disease. 284 is expressed by a large number of innate immune cells in intestinal lamina propria (LP). However, the role of 284 receptor in intestinal inflammation is still unknown.

Since 284 is highly expressed on some of lamina propria lymphocytes (LPL) in intestine, we hypothesized that 284 might play a crucial role for intestinal inflammation. Indeed, we found that 284 in expressed dendritic cells, macrophage, ILCs in intestinal lamina propri, and upregulated expression level after DSS administration. The progression and disease score of intestinal inflammation induced by DSS in 284- mice was much higher and faster than that observed in wild-type mice. At the cellular and tissue level, we found increased colonic inflammation and reduced intestinal epithelial cell proliferation, along with severely diminished number of dendritic cells in colon. Tissue repair related cytokines (IL-18, IL-1B) are significantly decreased in 284 deficient mice, and replenishing these cytokine could recover intestinal inflammation in 284 deficient mice group. Taken together, these data identify a novel function of 284 as a gate guard in intestinal inflammation in IBD.

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P.C6.01.12
ADSCs treatment hinders the advancement of fibrosis in liver in the early stage of NASH by reducing the IL17a-mediated inflammation

A. Nozzi, Y. Sakairi, M. Yamato1, K. Kagawauchi, T. Har, K. Yoshida, T. Komura, M. Takamur1, T. Wada1, M. Honda1, S. Kaneko1;
1System Biology, Kanazawa University, Kanazawa, Japan, 2Department of Gastroenterology, Kanazawa University Hospital, Kanazawa, Japan, 3Department of Cardiology, Kanazawa University Hospital, Kanazawa, Japan, 4Department of Laboratory Medicine, Kanazawa University, Kanazawa, Japan.

Non-alcoholic steatohepatitis (NASH) liver is defined by a steatotic/inflamed condition with advancing fibrosis, eventually becoming cirrhotic. There is no established therapy and pathogenesis is not completely uncovered. We demonstrated previously that adipose tissue derived stromal/stem cells (ADSCs) decreased fibrosis in NASH mice, now we investigated the mechanism between NASH and simple steatosis and, the effect of ADSCs treatment on early stage of NASH. C57Bl/6 mice were used for ADSCs isolation; simple steatosis or NASH was established in mice and hepatic inflammatory cells (HIC) isolated for DNA microarray analysis and cytokine secretion assay. Anti-Il17a or anti-CD4 antibody was administered weekly for 8 weeks, liver tissues isolated on week 9. Separately, ADSCs were administered on weeks 4 and 8, then liver collected on week 12. Fibrosis was assessed by AZAN staining, FACS and qRT-PCR. DNA microarray of HIC highlighted 868 up-regulated genes in NASH compared to simple steatosis, biological processes associated to CD4+ T cell immune response. Frequency of IL17-secreting cells in NASH-HIC was higher (37%) than control (15%). Anti-Il17a antibody decreased fibrosis (p<0.01), and down-regulated Col4a1 in NASH livers (p<0.05), anti-CD4 antibody was not effective. Administered ADSCs decreased il17a expression in NASH-HIC, reduced the IL17a-secreting cells population in HIC (25% compared to control (37%)), decreased fibrosis (p<0.01) and expressions of Col4a2 and Col1a1 in liver tissue (p<0.05). In conclusion, IL17a is involved in fibrosis development in NASH mice, and ADSCs administration reduced fibrosis by suppressing the IL17a-mediated inflammation in early stage of NASH.

P.C6.01.13
Differential innate immunity signature associated with disease activity in autoimmune diseases: systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis

A. Petrackova1, A. Smrzdova1, M. Schubertova1, M. Skacelova1, R. Fillerova1, V. Svatova2, K. Kawaguchi2, K. Radosny3, M. Kudelka2, F. Mrazek1, P. Horak1, E. Kringova1;
1Faculty of Medicine and Dentistry, Palacky University and Hospital Olomouc, Olomouc, Czech Republic, 2Technical University of Ostrava, Ostrava, Czech Republic.

Introduction: Mounting evidence indicates that innate immunity, especially Toll-like-receptors (TLR) and interleukin (IL)-1/IL-1R families, play essential roles in the pathogenesis of autoimmune diseases. The differential innate expression pattern associated with disease activity in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and systemic sclerosis (SSc) has not been established. Objective of the study was to elucidate the underlying differences in innate immunity signatures associated with disease activity in major autoimmune diseases.

Methods: We investigated gene expression of TLR1-10, 7 members of IL-1/IL-1R family, and interleukin 8 (IL-8/ICXCL8) in peripheral blood mononuclear cells from patients with autoimmune disorders taken at time of active disease: SLE (n=28, SLEDAI>6), RA (n=36, DAS28<2.3), and SSc (n=22, revised EUSTAR index<2.5) using high-throughput SmartChip Real-Time-qPCR system (WaferGen). Statistics were performed by R statistical software, P-value<0.05 was considered as significant.

Results: Differences of TLR7/8 expression and SSc by the upregulated expression of six genes (TLR3, TL7R, TLR8, TLR9, IL-1R8, and IL-1R1P1; p<0.05) and the significant down-regulated expression of IL-1R1 (p<0.05) was observed when compared to SLE and RA. In SLE, downregulated expression of IL-1R1 (p<0.05) was detected when compared to RA and upregulation of IL-1R8 (P=0.05) when compared to SSc. Conclusions: Innate immune gene expression signature in patients with autoimmune diseases in active disease stage was identified, showing high similarity between SLE and SSc. Grant support: MZ CR VS15-28659A, IGA UP_2018_016, MH CZ - DRO (FNOL, 00098892).

P.C6.01.14
Systemic inflammation induced by administration of TLR-7/8 agonist Resiquimod causes severe thrombocytopenia, myocardial erythrocyte accumulation and iron deposition in CFN mice

N. Baxan1, A. Papankilou1, I. Safles-Crawley1, R. Chowdhury1, O. Dubois1, N. Rosenthal1, L. Zhao1, S. E. Harding1, S. Sattler1;
1Imperial College London, Biological Imaging Centre, London, United Kingdom, 2Imperial College London, NHU, London, United Kingdom, 3Imperial College London, Centre for Haematology, London, United Kingdom.

Introduction: Topical application of the TLR-7/8 agonist Resiquimod has been suggested as inducible model of systemic lupus erythematosus. In response to Resiquimod administration, CFN (C57Bl/6J/FVBXkn) mice develop acute myocarids accompanied by myocardial haemorrhaging, followed by fibrosis and development towards inflammatory dilated cardiomyopathy. Importantly, we have shown previously that CFN mice show a degree of Resiquimod-induced cardiac damage which is significantly more severe than the levels observed in all three parental strains. Methods: CFN mice were treated with topical application of Resiquimod to the ear three times a week for a week. Magnetic resonance imaging was performed in vivo, blood collected for analysis of platelet count and hearts collected for histological analysis of inflammatory damage and fibrosis. Results: Magnetic resonance imaging shows a significant drop in T1, relaxation times (T1>1.7m, T2<0.7m) and T2* (P=0.014, n=3-5), localized primarily in the subepicardial regions of the intraventricular septum of Resiquimod-treated mice, suggestive of the presence of paramagnetic iron. This observation was confirmed by staining for iron in corresponding histology sections, which showed a significant iron accumulation (score: 0.096+/-0.07 and 1.36+/-0.22; P-value<0.005, n=3-10) in areas of low T2* values. We also detect severe thrombocytopenia in Resiquimod-treated CFN mice (platelet count/un 700+/-132x103 and 17+/-16x103; p-value 0.033, n=3), suggesting that cardiac erythrocyte accumulation may be caused by acute bleeding problems and that haemoglobin is the source of interstitial iron. Acknowledgements: This work was supported by the British Heart Foundation RM/13/1/30157 to SEH and PG/16/93/32435 to S.
POSTER PRESENTATIONS

P.C6.01.15 Investigating neutrophil impairment in patients with Neuromyelitis Optica
M. Schroeder-Castango1, S. Romero Suarez1, N. Borriconi2, F. Foul1, C. Infante Duarte2; 1Institute for Medical Immunology, Experimental Neuroimmunology, Charité - Universitätsmedizin Berlin, Berlin, Germany, 2NeuroCure Clinical Research Center NRCCR, Clinical Neuroimmunology, Charité - Universitätsmedizin Berlin, Berlin, Germany, 3NeuroCure Clinical Research Center NRCCR, Clinical Neuroimmunology, Charité - Universitätsmedizin Berlin, Berlin, Germany.

Introduction: There is recently published a meta-analysis showing that neutrophils in patients with Neuromyelitis Optica (NMO) have a reduced capacity to migrate towards chemotactic gradients compared to patients with Multiple Sclerosis (MS). This is in contrast to the observations in MS, neutrophils accumulate in NMO lesions and contribute to the damage cascade inside the CNS. However, there remains unclear, which factors lead to this accumulation. Defective neutrophil apoptosis accompanied by neutrophil activation promoting a pro-inflammatory environment has been already shown for other chronic inflammatory diseases. Thus, we here hypothesize that a generalized impaired cell death of NMO neutrophils might support their accumulation in inflammatory lesions.

Methods: We performed an assay to monitor death susceptibility of peripheral blood neutrophils from 20 NMO patients as well as 20 sex and gender matched healthy controls. Neutrophilic death was induced by incubating purified granulocytes with 25 mM PMA for 30 min at 37 °C. Cell death was analyzed by flow cytometry in CD16+ neutrophils using 7-ADD and Annexin V staining.

Results: Neutrophils from NMO patients show reduced cell death susceptibility compared to HC in our in vitro set-up.

Conclusion: Impaired cell death of neutrophil from NMO patients may contribute to neutrophil pathological accumulation in lesions. However, since the majority of the NMO patients are under immunomodulatory treatments, further evidences from untreated patients are needed to support this conclusion.

P.C6.01.16 Development and characterisation of a 4-dimensional in vitro model of ANCA-associated vasculitis
C. A. Wals1, N. Baus1, L. P. Erwig2, D. Kider2; 1Institute of Medical Sciences, Aberdeen, United Kingdom, 2Institute of Applied Health Sciences, Aberdeen, United Kingdom, 3NHS Grampian, Aberdeen, United Kingdom.

Introduction: ANCA-associated vasculitis (AAV) is a group of devastating autoimmune diseases affecting small/midsize blood vessels. The interaction of neutrophils and monocytes with the endothelium of blood vessels is key to understanding disease pathophysiology. There is limited knowledge about the temporal dynamics of these interactions and how they alter throughout the course of disease. We therefore aimed to develop a 4-dimensional in vitro model of AAV which would allow investigation of these crucial leukocyte-endothelial interactions.

Materials and Methods: Neutrophils and monocytes were isolated from peripheral venous blood collected from patients with AAV (Granulomatosis with Polyangiitis, Microscopic Polyangiitis and Eosinophilic Granulomatosis with Polyangiitis) and healthy donors. Using live cell spinning disc confocal microscopy, neutrophil/monocyte-human umbilical vein endothelial cell interactions were imaged in three dimensions for 3 hours. Video analysis software enabled quantification of leukocyte migration, adhesion, transmigration and degranulation.

Results: Degranulation and transmigration was significantly higher in neutrophils isolated from patients with active disease, compared to those in remission and healthy donors. Transmigration, rather than paracellular, transmigration of monocytes was significantly increased in the ANCA-positive patient cohort, compared to those who were ANCA-negative and healthy donors.

Conclusion: We have developed a novel 4-dimensional in vitro model of AAV encompassing several crucial leukocyte-endothelial functions at the forefront of disease pathophysiology. These preliminary experiments have highlighted key areas which are likely to be crucial in the development of the disease. Future work will include collecting longitudinal patient samples which will further verify the findings of this model.

P.C6.01.17 Epithelial IL-33 is regulated by the lymphoid stress-surveyance (LSS) response and confers protection against skin carcinogenesis
S. Ward1, R. Castro Seone1, G. Crawford1, M. Hayes1, J. Strid1; 1Imperial College London, London, United Kingdom.

Lymphoid stress-surveyance (LSS) refers to the capacity of tissue resident intraepithelial lymphocytes (IEL) to directly sense epithelial cell (EC) dysregulation and initiate a restorative response. γδTCR+ IEL in the skin are potent producers of IL-13 upon epithelial dysregulation, which is key to the LSS response and directly regulates EC function, promotes tissue homoeostasis and protects against skin carcinogenesis. This may be due to its prominent effect on skin EC, where IEL-derived IL-13 elicits a canonical EC stress-response with production of IL-33 mRNA. IL-33 is an abundant cytokine ‘alarm’ constitutively expressed in the nucleus of basal skin EC. Here we show that LSS regulates the level of EC IL-33 protein at steady state, as well as the release of IL-33 following topical exposure to the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA).

We also demonstrate that IL-33 protects against DMBA-induced epithelial carcinogenesis in the skin but not against subcutaneous tumour growth in a cell line-derived model. Furthermore, IL-33 restrains cellular infiltrate and epithelial hyperplasia during skin inflammation. This is possibly due to signaling via its receptor, ST2, on FoxP3 regulatory T cells (Treg), as ST2 is predominately expressed on the abundant Treg infiltrate in the DMBA-induced tumours and ST2-deficient mice are also more susceptible to inflammation-driven carcinogenesis. Together our data suggests that IL-33 acts via both the LSS response to tissue dysregulation and regulates both skin immunity, EC homeostasis and carcinogenesis.

P.C6.01.19 Regulatory Innate Lymphoid Cells in tissue homoeostasis and graft versus host disease
M. M. Shikhaeghi1, N. Haverkade1, Y. van Lieu1, B. Blom1, M. Hazenberg1; 1Amsterdam UMC, Amsterdam, Netherlands.

Background: Allergic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for patients with hematologic malignancies, including acute myeloid leukemia. Hematopoietic stem cell transplantation (allo-HSCT) recipients develop graft-versus-host-disease (GVHD). Damage of host tissues, such as the skin and gastrointestinal tract, caused by conditioning therapy are key in the pathophysiology of GVHD since release of danger-associated molecular patterns (DAMPs) activate allo-reactive immune responses. ILCs have an essential role in tissue homeostasis and -healing, for instance in the context of allo-HSCT and GVHD, due to their location at barrier surfaces and responsiveness to cytokines produced by activated cells in their local environment. DAMPs, such as extracellular ATP (eATP), interact with ectonucleotidases that are involved in the hydrolysis of eATP into the immunosuppressive metabolite adenosine. We studied ectonucleotidase expression on ILC and the role of eATP on ILC function.

Methods & Results: Using flow cytometry, we demonstrated that tissue-derived human ILCs, particular IL-22 producing ILC3 contributing to tissue healing, express ectonucleotidases on tissue and peripheral blood (PB) ILC were analysed by flow cytometry in CD16+ and CD16− neutrophils using 7-ADD and Annexin V staining.

Conclusion: We have developed a novel 4-dimensional in vitro model of AAV encompassing several crucial leukocyte-endothelial functions at the forefront of disease pathophysiology. These preliminary experiments have highlighted key areas which are likely to be crucial in the development of the disease. Future work will include collecting longitudinal patient samples which will further verify the findings of this model.

P.C6.02.01 Innate control of inflammation and tissue repair - Part 2
E. Bernaldo de Quirós1, R. Kennedy2, Y. Pérez3, Á. Hernández-Martí4, M. Campos5, P. Foul1, R. Correa-Rocha1; 1Laboratory of Immune-regulation, Gregorio Marañón Health Research Institute, Madrid, Spain, 2Pediatric Dermatology Division, Hospital Infantil Universitario Niño Jesús, Madrid, Spain, 3Pediatric Dermatology Division, Hospital Universitari Gregorio Marañón, Madrid, Spain.

Introduction: Ichthyosis is a heterogeneous group of genetic cutaneous disorders that alter the structure of the skin and where the immune mechanisms are not well elucidated. Specific therapies have not been identified and the treatment is currently symptomatic. Our aim is to identify the immunological profile in ichthyosis pediatric patients and determine the effect of ustekinumab (a monoclonal antibody that targets IL-12/23) on tissue and their immune system.

Materials and methods: We carried out an exhaustive analysis of immunological cell subsets by flow cytometry from peripheral blood (<3ml). Percentages and absolute counts of up to 75 subsets were analyzed.

Results: We have evaluated four samples of a 2 year-old male patient: one before starting the treatment with ustekinumab and the three others over 4 months. We observed high values of TCGR4+CD4+IL-17A-producing cells (Th17) before treatment. The frequency of Th17 was initially reduced by 30% associated with a clinical improvement of the patient. Although neither the Th17 frequency decrease nor the clinical improvement remained, we observed reduction in the itch.

Conclusions: We tried to evaluate a possible benefit of ustekinumab and draw a parallel between the frequency of Th17 lymphocytes and the clinical outcome. Even though we observed a clear improvement of the symptoms associated to a Th17 frequency reduction during the first month, this amelioration was not sustained over time. However, we proposed the use of Th17 frequency as a diagnostic and predictive marker for severity, as well as a follow-up marker in these patients in a larger study group.
Multiple sclerosis (MS) is considered a disease of T cell autoimmunity. However, cellular studies and genetic association studies indicate the importance of interactions with innate immune subsets in pathogenesis. For example, alterations in natural killer cell (NKC) phenotype or number have been correlated with MS disease.

Previous work in our laboratory identified two distinct subsets of CD56dim NKC, based on differential expression of CD95, CD8, CD27 and CD57. The NKC subsets were defined as double positive (DP) CD56++CD8+CD57+CD27- and triple negative (TN) CD56-CD8-CD57-CD27+. Beta-interferon treatment was associated with similar ratios of DP/TN subsets to those seen in healthy controls. Transcriptomic analysis showed that the TN subset expressed markers indicative of innate lymphoid cells (ILC3), confirmed by FACS. Individuals with MS in acute relapse had a higher proportion of the TN compared to the DP CD56++NKC subset.

Innate lymphoid cells are lineage-negative-cells lacking RAG-1-mediated rearranged receptors that react rapidly to changes in their micro-environment. Flow cytometric analysis of ILC3 cells in stable MS compared to acute relapsing MS patients and healthy controls has been carried out. Further analysis of selected populations using next generation RNA sequencing is underway to further understand the role of these cells in MS.

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P.C6.02.03

IL-17E participates in the recruitment of neutrophils in psoriasis skin inflammation


Introduction: IL-17E (IL-25) is over-expressed in psoriatic lesional skin, and dermal macrophages represent a main target of IL-17E. We aimed at understanding the role of IL-17E in psoriasis.

Methods: Macrophages were differentiated from peripheral blood monocytes of 5 healthy donors. Skin inflammation was provoked in BALB/c mice by tape-stripping or imiquimod application. Injection of rIL-17E or anti-IL-17E blocking antibodies was used to study IL-17E-dependent effects in vivo. Skin-infiltrating cells were profiled by multi-parameter flow cytometry and immunohistochemistry, and gene transcription modifications revealed by Nanostring and qPCR.

Results: In vitro, IL-17E induced the production of inflammatory mediators by human M3 macrophages through NFκB, p38 and STAT3 activation. Supernatants of IL-17E-stimulated macrophages contained high levels of IL-8 and enhanced the chemotaxis of neutrophils compared to supernatants of resting macrophages. p38-activation was required for these effects. In vivo, intradermal injection of rIL-17E in mice provoked a sustained inflammatory response immunofluorescence. The resulting inflammatory infiltrate was skewed towards a preferential recruitment of neutrophils in disfavor of T cells. The neutrophil chemokine CXCL1, along with IFN type I-related genes, TNFα and amphiregulin, were abundantly expressed in psoriatic skin lesions. Neutrophils infiltrating psoriatic skin expressed CXCR1 and CXCR2, the receptors for CXCL1. Noteworthy, IL-17E transcripts were upregulated in murine psoriatic skin in inflammation induced by tape-stripping and imiquimod, and neutralization of IL-17E led to significant reduction in the infiltration of neutrophils. Human lesional psoriatic skin, the number of IL-17E+ cells correlated with the number of neutrophils. Conclusions: Our data show that IL-17E favors the preferential recruitment of neutrophils in psoriasis skin inflammation and define a novel role for IL-17E in psoriasis.

P.C6.02.04

First steps towards a human in vitro 3D arthritic joint model

A. Damereau, A. Lang, M. Pfeiferberger, F. Buttgereit, T. Gabor

Charité - Universitätsmedizin Berlin, Berlin, Germany.

Our ultimate goal is to develop a valid human in vitro 3D arthritic joint model in order to simulate the pathogenesis of arthritis. The in vitro 3D joint model consists of different components in vivo. Using (1) the inflammatory component, (2) the cartilage and bone component, (3) the joint space with synovial fluid and (4) the synovial membrane, we have developed a model that can be used to study the inflammatory process in a realistic manner. This model consists of a monolayer of hMSC, formed on a polycarbonate membrane (synovial membrane component). Human bone marrow derived mesenchymal stromal cells (hMSC) are used to develop the different 3D tissue interactions between cells by cell contacts and signaling molecules. Our ultimate goal is to develop a valid human 3D arthritic joint model to study the immune mediated pathogenesis of arthritis using human material.

P.C6.02.05

Interaction of mesothelial CX3CL1 with monocyctic CX3CR1 promotes peritoneal fibrosis


Division of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.

Introduction: Monocytes and macrophages express the chemokine receptor CX3CR1. It has been ascribed differential roles in fibrosis development. Its ligand fractalkine (CX3CL1) was considered to be a mediator of chronic peritoneal fibrosis induced by sterile, pyrogen free dialysis solution. Mesothelial cells expressed its ligand CX3CL1, and CX3CL1 in this process has not been described.

Methods: Fibrosis development and CX3CR1 expression were investigated in a murine peritoneal dialysis model in vivo and in murine and human primary cells in vitro by histology, and CX3CR1 expression was confirmed in the murine mesothelial HMLE cell line. The interaction of CX3CL1 with monocyctic CX3CR1 induces peritoneal fibrosis in vivo and in vitro.

Results: CXCL1 expression increased in chronic peritoneal fibrosis in vivo and in vivo CD11b+ monocyctic cells in vitro during co-culture with fibroblasts. We identified the pro-fibrotic cytokine TGFβ as a mediator. CX3CR1-CX3CL1 interaction as a pro-fibrotic pathway in peritoneal fibrosis.

Conclusions: Our data identify CX3CR1-CX3CL1 interaction as a pro-fibrotic pathway in peritoneal fibrosis.

P.C6.02.06

Alarmin IL33/ST2 axis function during mucosa inflammation and cancer

M. A. HERMOSO1, K. Dubois1, M. De la Fuente1, G. Landskron2, D. Diaz-Jimenez1, D. Simian1, R. Quera3

1Facultad de Medicina, Santiago, Chile, 2Clínica Las Condes, Santiago, Chile.

The Interleukin-33 (IL33)/ST2 axis has been noted in numerous diseases, including asthma, rheumatoid arthritis, inflammatory bowel diseases and, more recently, in cancer and Alzheimer’s disease. IL33, a member of the IL1 cytokine family is mainly associated with the induction of T-helper 2 (Th2) and alternative macrophage M2 immune responses through ST2 receptor (encoded by the IL1RL1 gene). It is expressed as both a membrane-anchored receptor (ST2L) activated by IL33 and as a soluble receptor (sST2) with anti-inflammatory properties. Here we showed that during pathological conditions of the intestinal mucosa, such as in ulcerative colitis (UC) or colon cancer (CRC), levels of IL33 and sST2 are increased in tissue and the periphery. In UC, serum/mucosal ST2, and fecal calprotectin (FC) content correlated with clinical/endoscopic activity of patients, becomes a useful biomarker in the clinical practice. Also, IL33 content in left-sided CRC increases in patients with lymphatic metastasis and its tumor localization is associated with abundant desmoplasia. IL33 transcript levels from CAFs directly correlate with the capacity to induce cell migration and a mesenchymal phenotype in CRC cell lines, suggesting that IL33 ST2 mediates processes associated with invasion and interaction, specifically between CAFs and epithelial tumor cells. We also aimed to demonstrate that single-nucleotide polymorphisms (SNPs) in IL1RL1 appear to be associated with gene expression regulation in UC patients. Finally, we propose that the IL33-ST2 system in innate and adaptive immunity regulates cellular and molecular mechanisms, thus impacting on mucosal inflammatory disorder treatment and aiding prognosis. Fondecyt 1170648.
In healthy brain, leukocyte infiltration into the central nervous system (CNS) is limited by the blood-brain barrier (BBB). In multiple sclerosis (MS), this tightly regulated immune surveillance is hampered, leading to infiltration of myelin-specific T-cells into the CNS parenchyma. Oncostatin M (OSM) is produced in lesions of MS patients and we demonstrated in previous research that OSM protects demyelination and enhances neurite outgrowth. Here, we hypothesize that OSM also has a protective role in CNS damage via the BBB. Recently, we demonstrated a decrease (p=0.05) in vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression (mRNA and protein) on mouse BBB endothelial cells after OSM treatment under inflammatory conditions. mRNA levels of tight and adherens junctions were not affected, although the effect on the protein level needs to be examined. To investigate the effect of OSM on the BBB in vivo, experimental autoimmune encephalomyelitis (EAE) was induced in OSMR KO animals. OSMR-deficiency leads to a reduced disease score (p=0.0351, F(1,42)=4.743) during the acute phase. In contrast, tissue analysis revealed a persistent inflammatory environment in the chronic phase of EAE in OSMR KO mice. In the in vitro data imply a protective effect of OSM on the BBB, while the in vivo data suggest both a disease promoting effect in the acute phase and a disease limiting effect in the chronic phase of EAE. Additional in vitro and in vivo experiments are necessary to reveal the true role of OSM on the BBB.

P.C6.02.09
Association of NLRP3 single nucleotide gene polymorphisms with the susceptibility to Relapsing-Remitting Multiple Sclerosis

M. Izod, O. Imani, A. Azimi, Z. Salehi;
Medical Faculty, Tehran, Iran, Islamic Republic of.

NLRP3 inflammasome is a multi-protein complex that controls production of pro-inflammatory cytokines, IL-1β and IL-18, through caspase-1 activation. These inflammatory cytokines play an important role in the development of multiple sclerosis (MS). The inflammasome NLRP3 gene variations and expression level have been suggested to affect the immune system activity. In this case-control study we determined the association of NLRP3 genetic variants and expression with MS. We analyzed four common single nucleotide polymorphisms (SNPs) of NLRP3 (rs10754558, rs358294, rs3806265, rs4612666) in a group of 150 Iranian patients with relapsing remitting MS (RRMS) in comparison to 100 healthy controls using the TaqMan method. For analysis of NLRP4gene expression level, we studied a group of 37 RRMS patients (18 patients at relapse phase and 19 remission phase, treated with IFN-β) in comparison to 22 healthy controls using Realtime PCR. In this study, we found that NLRP3 rs3806265 C allele and CC genotype were significantly more frequent in the RRMS patients. While, the frequency of T allele significantly decreased in controls (P=0.05). The frequency of CG genotype at position ns10754558 was also significantly higher in the controls compared with patients (P=0.03). Moreover, expression level of the NLRP3 in patients at remission phase was significantly reduced in comparison with patients at relapse phase and also healthy controls (P=0.01 and P=0.04, respectively). The association of NLRP3 polymorphisms with the susceptibility of MS and reduced its expression after IFN-β therapy support the idea that NLRP3 inflammasome could have a critical role in inflammatory responses in MS.

P.C6.02.10
The link between angiogenesis and osteogenesis in spondyloarthritis

M. H. Koo1, J. H. van Hambregt2, G. Kollia3, D. L. Borton4, L. M. van Duivenvoorde4, S. W. Tso2;
1Department of Experimental Immunology, AMC/University of Amsterdam, Amsterdam, Netherlands, 2Department of Rheumatology & Immunology Center, AMC/University of Amsterdam, Amsterdam, Netherlands, 3Division of Immunology, Biomedical Sciences Research Center Alexander Fleming, Vari, Greece, 4Department of Physiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece.

Background: Spondyloarthritis is characterized by inflammation, extensive angiogenesis and pathological osteogenesis. Transmembrane (tm)TNF transgenic (tg) mice that overexpress tmTNF exhibit features of spondyloarthropathies including chronic inflammation and pathologic features of osteoarthritis. tmTNF ligation to TNF receptor 2 in endothelial cells (ECs) can induce signal transduction pathways, that may promote these processes. Of note, angiogenesis and osteogenesis are coupled by EC differentiation towards a type H (CD34+endo2+end3−) phenotype. We investigated the link between pathological angiogenesis, inflammation and osteogenesis in tmTNF tg mice.

Methods: Vertebrae from 6 and 12 weeks and 8 months old tmTNF tg mice or non-tg littermates (n=18) were prepared by cutting 60 µm thick cryosections for confocal imaging.

Results: tmTNF tg mice exhibited ectopic osteogenesis which was not observed in non-tg littermates. Immunostainings showed that type H vessels are in the vicinity of the ectopic osteogenesis and osteo-synoviocytosis. Furthermore, there is increased osteogenesis, type H vessel presence and a different vessel architecture within the vertebrae of tmTNF tg mice compared to non-tg littermates that progresses with age. Non-tg littermate vertebrae only have physiological osteogenesis, which is in the metaphysis and periosteum. In addition, tmTNF tg mice exhibit altered bone marrow (BM) architecture containing extensive lymphoid aggregates, which predominantly consisted of B220+ aggregates and contain high endothelial venules.

Conclusions: tmTNF overexpression in mice leads to development of type H vessels associated with ectopic osteogenesis. In addition, extensive lymphoid aggregates develop in the BM. Current studies are aimed at identification of signaling pathways in ECs that contribute to these processes.

P.C6.02.12
Targeting EphA signaling by dasatinib inhibits intestinal inflammation

A. Kim, S. Shim, S. Park, J. Myung;
Korea Institute of Radiological & Medical Science, Seoul, Korea, Republic of.

Irradiation functions a crucial role in the pathogenesis of intestinal inflammatory diseases, which regulated by vascular permeability and leukocyte infiltration. Thus, a potential therapeutic strategy for the treatment of radiation-induced inflammatory diseases would be assays to target endothelium cells that could induce the regulation of vascular barrier and leukocyte extravasation at sites of inflammation. In the present study, we demonstrated that the clinically approved cancer drug dasatinib, a tyrosine kinase inhibitor, to control inflammation. To evaluate whether irradiation affects immune cell activity, we exposed to irradiation in human or mice-derived endothelial cell and leukocyte. Irradiated HUVEC showed increased monocyte, macrophage and neutrophil infiltration in lamina propria of mice. However, HUVEC treated with dasatinib decreased inflammatory cytokine level and adhesion to leukocyte through inhibition of p-VE-cadherin and ICAM. We next investigate what molecular signaling was regulated by dasatinib. We showed that irradiation triggered EphA signalling, inducing inflammatory effects whereas dasatinib reduced phosphorylated EphA. Moreover, decreased EphA signalling can inhibit inflammation-associated cytokine and adhesion molecules in HUVEC. Treatment with dasatinib inhibits irradiation-induced vesel permeability and leukocyte infiltration in vivo. Therefore, irradiation-induced damage of endothelial and leukocyte activates inflammatory response but treatment with dasatinib could usefull strategy for reducing inflammation.

P.C6.02.13
Synovial osteoprogenitor phenotype in patients with rheumatoid arthritis

1Department of Orthopedic Surgery, University Hospital Center Zagreb, University of Zagreb School of Medicine, Zagreb, Croatia, 2Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia, 3Laboratory for Molecular Immunology, University of Zagreb School of Medicine, Zagreb, Croatia, 4Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia.

Introduction: Rheumatoid arthritis (RA) is a chronic, autoimmune joint inflammation, which results in disability due to irreversible joint destruction. Current treatments can slow the progression of the disease, but are still ineffective in a number of individuals and mainly target inflammation. Since there is increasing evidence on the ability of mesenchymal cells to promote regeneration and suppress inflammation, we focused on the phenotype of non-hematopoietic progenitor populations in synovial tissue of patients with arthritis.

Materials and methods: We analyzed cellular composition of synovial tissue from 9 RA patients undergoing surgery, and 6 control patients undergoing arthroscopic treatment. Cells were released by collagenase digestion and analyzed by flow cytometry after labeling with two panels: 1. CD3, FITC, CD4, CD8, CD105, CD14, CD19, APC-CD71, CD235a-APC, CD41-APC, CD42a-APC. Results: Synovial infiltrate in RA had higher proportions of CD8+ and CD19+ cells, and similar variable proportions of CD11b+ and CD14+ in comparison to control samples. Amongst non-hematopoietic (CD45+CD31+CD235a+) cells, proportion of CD105+ cells was significantly increased in RA patients, whereas proportion of CD200+ cells was similar to controls. Amongst CD200+ cells, CD200+CD105+ population was more abundant, while CD200−CD105+ cell numbers were reduced in RA samples in comparison to healthy controls. Conclusions: There are significant differences in the composition of synovial non-hematopoietic compartment between RA patients and healthy synovia. According to experimental studies, CD200+CD105+ cells are considered as earliest osteoprogenitors, and also implicated in regulation of myeloid cell accumulation and activity. Loss of these cells might favor inflammation and arthritis progression.
Sphingosine-1-phosphate is an immune-modulating lipid that has been shown to influence the immune response through five G-protein coupled receptors. For instance, migration of T lymphocytes into the circulation depends on sphingosine-1-phosphate receptor 1 (S1PR1). The less well understood S1PR4, primarily expressed on immune cells, has been found to modulate inflammatory responses but whether it is also involved in migration of immune cells is currently discussed. There is some indication that it is involved in neutrophil homing and potentially trafficking. In an imiquimod-induced mouse model of psoriasis we observed that a knockout of S1PR4 reduces inflammation, accompanied by a reduced infiltration of neutrophils and macrophages into the psoriatic lesions. At the same time we found a reduction in pro-inflammatory cytokines and chemokines such as IL-6, CCL2, and CCL13. Similar data were also obtained in a model of Zymosan-induced peritonitis. This suggests a role of S1PR4 in the early stages of inflammation by promoting neutrophil and macrophage trafficking via chemokine production. Using RNAseq in mouse tissues and in vitro signaling pathway analysis, we identify the cellular source of these chemokines and the pathways downstream of S1PR4 that regulate their expression. Together, our data provide a novel feature of inflammation regulation by S1P and S1P receptors.

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**P.C6.02.14**
Modulation of immune cell infiltration by S1PR4
C. Ringel, B. Bruene, A. Weigert; Institute of Biochemistry I, Frankfurt am Main, Germany.

**P.C6.02.15**
Adipocyte function in the mesenteric fat tissue depends on the innate immune system
F. Schmidt; 1 University Medical Center Utrecht, Utrecht, Netherlands, 2 Sint Maartenskliniek, Nijmegen, Netherlands.

In Crohn’s disease the mesenteric fat tissue is creeping around the inflamed intestine. Previous data indicate that adipocytes express functional receptors of the innate immune system. Thus, translocating bacteria might serve as trigger for this unique finding. To address this question MyD88+ and adipocyte-specific MyD88 knockout (MyD88AdipoqCre) mice were studied. MyD88+ but not MyD88AdipoqCre mice presented with an increased mortality upon dextran sodium sulfate (DSS)-treatment. The effector response in the mesenteric fat was reduced, paralleled by an increased bacterial translocation. MyD88AdipoqCre mice revealed less severe disease as indicated by lower concentrations of IL-6 and TNF in ex vivo colonic tissue supernatants and higher expression of IL-10 in the mesenteric fat.

Creeping fat is characterized by a strong cellular infiltrate. To determine phenotypic and functional differences of the mesenteric fat tissue between MyD88+, MyD88AdipoqCre and wildtype (WT) mice, cellular composition as well as chemotactic function was analyzed. In health, no differences with regard to macrophage and T cell populations were observed. To assess chemotactic function of the mesenteric fat tissue, transmigration assays were performed. WT or MyD88AdipoqCre+ fat did attract monocytes whereas however, LPS-stimulated fat from AD T cells. However, LPS-stimulated fat from AD T cells. However, LPS-stimulated fat from AD T cells. However, LPS-stimulated fat from AD T cells. However, LPS-stimulated fat from AD T cells. However, LPS-stimulated fat from AD T cells. However, LPS-stimulated fat from AD T cells. However, LPS-stimulated fat from AD T cells. However, LPS-stimulated fat from AD T cells. However, LPS-stimulated fat from AD... INdicated that collagen type I of bone marrow-derived macrophages shifted to an anti-inflammatory treatment with either WT or MyD88AdipoqCre+ fat. These data indicate that adipocytes contribute to the milieu in the mesenteric fat with regard to cellular infiltrate as well as local mediators and depend on the innate immune system.

**P.C6.02.16**
Increased intra-articular granzyme M and local proinflammatory cytokine release in Rheumatoid Arthritis
L. Shan, 1,3 A. C. Wensink, 1,2,4 L. L. van den Hoogen, 1,2 J. Meeldijk, 1 H. M. Kok, 1,2 L. H. Jongeneel, 4 M. E. van Marum, 1,2 A. C. Wensink, 1,2 J. A. van Rooin, 1,2,4 N. Bovenschen
1 National Institute for Public Health and the Environment, Bilthoven, Netherlands, 2 Sint Maartenskliniek, Nijmegen, Netherlands, 3 Immunology department, Landskapi - The National University Hospital of Iceland, Reykjavik, Iceland, 4 Center for Rheumatology Research, Landskapi - The National University Hospital of Iceland, Reykjavik, Iceland.

**Objective:** Granzymes are serine proteases involved in eliminating tumor cells and virally infected cells. In addition, extracellular granzyme levels are elevated in inflammatory conditions, including several types of infection and auto-immune diseases, such as rheumatoid arthritis (RA). While GrA and GrB have been associated with RA, for a role the other three granzymes (GrH, GrK and GrM) in this disease remains unclear. Here, we investigated the presence and role of GrM and GrK in serum and synovial fluid of patients with RA, psoriatic arthritis, and osteoarthritis.

**Methods:** Grzyme levels were determined in serum, synovial fluid, peripheral blood mononuclear cells (PBMCs) and synovial fluid mononuclear cells (SFMCs) of RA patients and relevant control groups. In addition, the link between GrM and other inflammatory cytokines in synovial fluid was investigated.

**Results:** Serum GrM and GrK levels were not affected in RA. GrM, but not GrK levels were elevated in synovial fluid of RA patients. GrM was mainly expressed by cytotoxic lymphocytes in SFMCs with a similar expression pattern as compared with PBMCs. Intra-articular Gr/M expression correlated with IL-25, IL-29, XCL1, and TNFα levels. Intriguingly, purified GrM triggered the release of IL-29 from human fibroblasts in vitro.

**Conclusions:** These data indicate that GrM levels are increased in RA synovial fluid and that GrM can stimulate proinflammatory IL-29 release from fibroblasts, suggesting a role of GrM in the pathogenesis of RA.

**P.C6.02.17**
The anti-microbial peptide LL-37 shapes the response of keratinocytes to psoriasis related stimulations
H. Sigurjónsdóttir1,2, J. Freydot1,2, B. R. Lúdvíksson1,2; 1 University of Iceland, Iceland, 2 Landspitali - The National University Hospital of Iceland, Reykjavik, Iceland.

**Objective:** Psoriasis is a common inflammatory skin disease that is characterized by infiltration of immune cells into the skin, hyperproliferation of keratinocytes in the basal layer of the epidermis and an imbalanced cytokine and chemokine environment. LL-37 is an anti-microbial peptide that is a part of the innate immune system. It is upregulated in psoriatic patients, both in blood and skin. The main goal of this research was to map out the effects that LL-37 has on the secretion of keratinocytes. Material and methods: A keratinocyte cell line and primary keratinocytes were cultured and stimulated with psoriasis related stimulations or left unstimulated, all in the presence or absence of LL-37. Cell supernatant was collected and analysed with Luminex for 27 soluble analytes. Results: All chemokines, cytokines and growth factors were measured. The main conclusion was that LL-37 has a pro-inflammatory effect on the secretion of keratinocytes. LL-37 increases secretion of some analytes and increase secretion of other analytes. Keratinocytes were shown to produce IL-17A but the addition of LL-37 did not affect the secretion. Other cytokines and chemokines, such as CCL2 and CXCL1 were increased. Conclusion: LL-37 is a pro-inflammatory cytokine that increases the secretion of keratinocytes.

**P.C6.02.18**
Au nanorod-induced NLRP3 inflammasome activation is mediated by ER stress
R. J. Vandebril1, S. Remy1, J. Vermeulen1, E. Hurkmans1, N. Bastu1, B. Pelaz2, U. Punter2, W. Para3, J. Pennings1, i. Nelissen1; 1 National Institute for Public Health and the Environment, Bilthoven, Netherlands, 2 VITO NV, Mol, Belgium, 3 Institut Catala de Nanociencia i Nanotecnologia (ICN2), Barcelona, Spain, 4 Philips Universitaet, Marburg, Germany.

The widespread and increasing use of engineered nanomaterials (ENM) increases the risk of human exposure, generating concern that ENM may provoke adverse health effects. In this respect, their physicochemical characteristics are critical. The immune system may respond to specific ENM properties by inflammatory reactions. Inflammasome activation has drawn significant attention since inflammasomes, especially NLRP3 respond to a wide range of stimuli including nanoparticles, and their activation is associated with various inflammatory diseases, including lung fibrosis, obesity and type-2 diabetes. Inflammasomes are intracellular multiprotein complexes that assemble upon stimulation, resulting in activation of caspase-1 that in turn induces production of IL-1B and IL-18, which are potent mediators of inflammation. Endoplasmic reticulum (ER) stress has been reported as one of the mechanisms underlying NLRP3 inflammasome activation.

In this study, PEGylated Au ENM of ~60 nm, having different shapes (stars, spheres and rods) were extensively characterized, and tested for possible LPS contamination. PMA- activated THP-1 cells were exposed to these, and cell viability and IL-1B production were measured to assess NLRP3 activation. In addition, the exposed cells were subjected to transcription analysis using microarray analysis to investigate related signaling pathway regulation. PEGylated Au nanorods (NR), but not nanostars or nanospheres, showed NLRP3 inflammasome activation. Cells deficient in the NLRP3 scaffold or ASC adaptor did not show this effect. Only NR-exposed cells showed down-regulation of ER-associated cholesterol metabolism.

This may suggest that ER stress mediates NLRP3 inflammasome activation by Au NR. Supported by the EU funded project FutureNanoNeeds (Grant agreement N° 604602).
Our study included 228 RA patients (age: 47±13 years; Sex Ratio: 1:6; Disease duration: 9.4±8.2 years) compared to 188 healthy controls (age: 35±10 years; Sex Ratio: 1:2). Genomic DNA from RA patients and controls was genotyped, using TaqMAN technology, for VEGF-2578A/C, -1154A/G and -634C/G SNPs. Allele and genotype frequencies were markedly expressed in response to lipo polysaccharides (LPS). We further demonstrate alleles of rs6897932, a non-synonymous IL7R polymorphism associated with susceptibility to Multiple Sclerosis, Ankylosing Spondylitis and Primary Biliary Cirrhosis, form the key determinant of both surface IL7R and sIL7R in the context of inflammation. No effect of this allele was observed in unstimulated monocytes or in other lymphoid cells. Monocyte-derived sIL7R was greatly in excess of that produced by CD4 T-cells, and strongly associated with both rs6897932 genotype and expression of the splicing factor gene, DXD58A. Stem-cell-derived monocytes are sensitive to exogenous IL-7, which elicits a defined transcriptional signature, flow cytometry and single-cell sequencing of synovial fluid derived monocytes from patients with Spondyloarthritis shows an enlarged subset of IL7R+ monocytes with a unique transcriptional profile that markedly overlaps the in-vitro IL-7 induced gene expression signatures. This data demonstrate disease-associated genetic variants at LILRA7 specifically impact monocyte protein IL7R and sIL7R following innate immune stimulation, suggesting a previously unappreciated key role for monocytes in IL-7 pathway biology and IL-7 associated diseases.

Our findings show that N. brasiliensis infection alters the gut microbiota composition, resulting in a general increase of bacteria belonging to phyla Firmicutes, Bacteroidetes and Actinobacteria, in particular orders; Clostridiales, Bacteroidales and Coriobacteriales. We here demonstrated that infection with the parasitic nematode Nippostrongylus brasiliensis significantly reduced body weight, fasting blood glucose and oral glucose tolerance test in mouse models of T2D. We also found that this infection was associated with boosted type 2 immune responses measured by an increase in the eosinophil number. We further investigated the effect of this helminth infection on the gut microbiota composition. Interestingly, we found that N. brasiliensis infection altered the gut microbiota composition, resulting in a general increase of bacteria belonging to phyla Firmicutes, Bacteroidetes and Actinobacteria, in particular orders; Clostridiales, Bacteroidales and Coriobacteriales.
RNA and histones in dead cells synergistically provoke FSAP activation in serum

L. Bulder1, G. Marsman2, F. Stephan2, B. Lukan3, S. Zeerleder1,2
1Sanquin Research, Dept of Immunopathology and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, 2Department of Hematology, Academic Medical Center, Amsterdam, Netherlands.

The plasma serine protease Factor VII-activating protease (FSAP) has been implicated in thrombosis and vascular remodeling. Moreover, FSAP is crucially involved in the release of chromatin from dead cells. FSAP circulates in an inactive form and is activated upon contact with late apoptotic or necrotic cells. FSAP binds to purified negatively charged molecules, and positively charged nucleic acids, e.g. histones, which have both been implicated in the auto-activation of FSAP albeit through different mechanisms. We aimed to identify the component(s) from dead cells that mediate FSAP auto-activation in serum. Binding of FSAP to late apoptotic Jurkat cells was studied using confocal microscopy and flow cytometry. Digestion of RNA in late apoptotic cells markedly reduced the binding of FSAP to these cells, and concurrently the activation of FSAP induced by these cells was also reduced. In contrast, DNA digestion strongly enhanced both the binding and activation of FSAP. Upon cellular fractionation, the cytoplasmic fraction containing mRNA did not induce FSAP activation, whilst the nuclear fractions that predominantly contained histones did induce the activation of FSAP. In serum, the addition of histones induced FSAP activation, whilst activation did not occur upon addition of RNA. However, when RNA was combined with histones this markedly enhanced the activation of endogenous FSAP in serum. Our results indicate that both RNA and histones are involved in the activation of FSAP by dead cells. RNA does not directly induce the auto-activation of FSAP, but may promote/facilitate auto-activation of FSAP induced by histones.

An immunosuppressive tick salivary gland protein DsCystatin interferes with Toll-like receptor signaling by downregulating TLR6

J. Das
Institute of Biological and Medical Sciences, Soochow University, Suzhou, China.

Ticks, blood-feeding arthropods, secrete immunosuppressive molecules that inhibit host immune responses and provide survival advantages to pathogens. In this study, we characterized the immunosuppressive property of a novel tick salivary protein, DsCystatin, from Dermacentor silvarum of China. DsCystatin directly interacts with human Cathepsin L and B and inhibited their enzymatic activities. DsCystatin impaired the expression of inflammatory cytokines such as IL1β, IFNγ, TNFα and IL6 from mouse bone marrow derived macrophages (BMDMs) that stimulated with LPS or Borrelia burgdorferi. Consistently, DsCystatin inhibited the activation of mouse BMDMs and bone marrow-derived dendritic cells (BMDCs) by downregulating the surface expression of CD80 and CD86. Mechanically, DsCystatin inhibited LPS or B. burgdorferi induced NFκB activation. For the first time, we identified that DsCystatin attenuatedTLR4 signaling by targeting TLR6. DsCystatin enhanced LPS induced autophagy, mediated TLR6 degradation via an autophagy dependent manner, thereby impeded the downstream phosphorylation of idola and the nuclear transport of NFκB. Finally, DsCystatin relieved the joint inflammation in B. burgdorferi or C. burnetii infected’s adjacent induced mouse arthritis models. These data suggest that DsCystatin is a novel immunosuppressive protein and can be potentially used in the treatment of inflammatory diseases.

Signaling through purinergic receptor P2Y2R enhances macrophage IL-1beta production

G. de la Rosa, A. Gómez, P. Pelegín
IMIB-Arrixaca, El Palmar, Murcia, Spain.

Release of nucleotides during processes such as necrosis or apoptosis have been described to have both proinflammatory and antiinflammatory effect on the surrounding cells. Purinergic receptors expressed sentinel macropoles will be able to modulate the inflammatory response depending on the context. Here we describe how ATP and ATP-dependent IL-1βeta production during Lipoopolysaccharide(LPS)-induced murine resident peritoneal macrophage activation. Inhibition of the purinergic receptor P2Y2R with AR-C 139852x significantly blunted the phosphorylation of P65-NF-κB at Ser 536 without altering the phosphorylation of its inhibitor IκBα in the lungs. The elastase-induced expression of NF-κB dependent IL-1β and TNFα was reduced. Like PARP-1, AR-C 139852x reversibly induced autophagy, mediated TRAF6 degradation and autophagy dependent NFκB phosphorylation. These findings provide a novel unique proinflammatory signature might be relevant in future inflammation studies.

C-reactive protein promotes inflammation through metabolic reprogramming of human macrophages

M. Neuling1, L. Sthiran2, A. van der Ham3, M. van Weeghel3, D. Baeten2, M. van Weeghel2, A. S. Sanz Bartolome4, J. van Den Dunnen5, J. van Attikum6, B. Everts7, L. de Boer8, L. Sritharan2, M. van Weeghel3, A. van der Ham3, B. Luken1
1AMC, Amsterdam, Netherlands, 2UMC, Leiden, Netherlands.

C-reactive protein (CRP) is an acute-phase protein produced in high quantities by the liver in response to infection and during chronic inflammatory disorders, and is therefore widespread in the human population. Although CRP is known to facilitate the uptake of dead cells and particular strains of bacteria by phagocytic cells, it still remains largely elusive whether CRP displays additional immunological functions. Strikingly, we here provide evidence that CRP is not only a marker, but also a cause of inflammation by strongly amplifying pro-inflammatory cytokine production. We show that cells (BMDM) that were conditioned by CRP, as a result of binding to its ligand phosphocholine on dead cells or bacteria, but not soluble CRP, strongly enhances TNF, IL-1β, and IL-23 production by human macrophages. While CRP does not induce cytokine production individually, CRP synergizes with particular pattern recognition receptors such as TLR-like receptors (TLRs) to amplify cytokine gene translation. Based on the specificity of blocking antibodies we identified Fc gamma receptor Ia and Ila (FcγRI and FcγRIIa) as the main receptors responsible for initiating CRP-induced inflammation. Furthermore, we demonstrate that the increased production of pro-inflammatory cytokines was dependent on signaling through kinases Syk, PI3K, and Akt, resulting in enhanced pro-inflammatory cytokine production through metabolic reprogramming, particularly through amplified glycosylation, amplified fatty acid synthesis, and strongly reduced oxidative phosphorylation.

These data indicate that CRP-induced metabolic reprogramming provides a novel mechanism of host defense against bacteria, but may also exacerbate pathology in the context of various CRP-associate inflammatory disorders, including rheumatoid arthritis and atherosclerosis.

Potential beneficial effects of Poly (ADP-ribose) polymerase-1 inhibition in COPD pathogenesis

V. Dhawale, A. S. Noura
Department of Biochemistry, Panjab University, Chandigarh, India.

Chronic obstructive pulmonary disease (COPD) is one of the leading causes responsible for global morbidity and mortality, and has no effective treatment available till date. We have previously reported that PARP-1 plays a crucial role in the establishment of airway inflammation associated with asthma/acute lung injury. In the present work, we have evaluated the beneficial effects of PARP-1 inhibition on COPD pathogenesis utilizing elastase-induced mouse model of the disease. Our data show that PARP-1 inhibition by olaparib (5mg/kg i.p.) significantly reduced the elastase induced recruitment of inflammatory cells particularly neutrophils. Reduction in the lung inflammation was associated with suppressed myeloperoxidase activity and restoration of redox status in the lung tissues towards normal. Further, the normalization of redox status in lungs was coupled with suppressed PARP-1 activity as reflected by reduced ribosylation of tissue proteins. Western-blot analysis showed that olaparib administration prior to elastase instillation blunted the phosphorylation of P65- NF-κB at Ser 536 without altering the phosphorylation of its inhibitor IκBα in the lungs. The elastase-induced expression of NF-κB dependent pro-inflammatory cytokines (TNF-α, IL-6), chemokine (MIP-2), and growth factor (GCSF) was down-regulated severely both at the mRNA and protein levels upon olaparib administration. A significant protection against the elastase-induced emphysema was also observed. Additionally, it was observed that PARP-1 heterozygosity has similar effects as olaparib treatment with specific blocking antibodies. PARP-1 inhibition of lung tissues was associated with significantly reduced levels of pro-inflammatory cytokines and chemokines, and reduced lung inflammation.

ATF4 and XBPI regulate the expression of ULBP1 in kidney cells upon endoplasmic reticulum stress activation

P. Diaz Bulnes1, A. S. Sanz Bartolome3, A. Baragallo Raneros2, R. M. Rodriguez2, C. López Lorrez2, B. Suarez-Alvarez2
1Traslational Immunology Laboratory, Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain, 2Laboratory of Nephrology, IIS-Fundacion Jimenez Diaz, Madrid, Spain.

Acute kidney injury may result from a variety of processes such as hypoxia, inflammation and proteinuria that lead to development and progression of kidney disease. As consequence, dysfunction of Endoplasmic Reticulum (ER) is triggered causing the activation of unfolded protein response (UPR). Expression of NGK202 is ligands in damaged kidneys can induce the activation of cytotoxic cells (NK and CD8+ T) through the interaction with NGK202 receptor. Our aim was to a analyze the influence of ER stress on the NGK202 expression during the induced kidney damage. Activation of UPR by tunicamycin and thapsigargin in human and mouse renal tubular cell lines induces the expression of protein sensors (ATF6, PERK and IRE1a) that initiate the three major signaling pathways of UPR. Moreover, that correlates with a significant increased of human ULBP1 ligand and its murine homologous MUL1. Similar results were observed in experimental renal damage models (ATK / JUO) where UPR activation is associated with increased MUL1 expression.

416

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Activation with specific inducers of each signalling pathway and gene silencing assays showed that PERK (ATF4) and IRE1α (XBP1) are directly involved in the ULBP1 and MULT1 expression. Inactivation of PERK (ATF4) and IRE1α (XBP1) by siRNA transfection decreased the mRNA expression of ULBP1 and MULT1 in both adherent and non-adherent cells, but increased cell death. These findings suggest that activation of the PERK and IRE1α pathways plays a crucial role in the regulation of ULBP1 and MULT1 expression.

Conclusion: The present study demonstrates that PERK and IRE1α pathways are involved in the expression of ULBP1 and MULT1 and play a critical role in the regulation of cell death in keratinocytes. These findings provide new insights into the molecular mechanisms underlying the interaction between keratinocytes and lymphocytes in the context of antiviral defense and highlight potential therapeutic targets for the treatment of infectious diseases.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
We also found that ConA administration led to an increase in the retinoic acid early-inducible 1 (RAE-1) surface expression on wild type hepatocytes. Finally, we found that ConA has no effect on FAS-L expression or cytokine production by γδ T-lymphocytes, and thus propose that NKG2D-L expression on stressed hepatocytes promote cytotoxic activity of γδ T-lymphocytes via its interaction with NKG2D contributing to hepatic injury. Conclusion: our results highlight NKG2D as an essential receptor required for activation of the γδ T-lymphocytes in Con A-induced hepatitis and indicate that it represents a potential drug target for prevention of autoimmune hepatitis.

P.C6.04.02
Role of neutrophils in the Imiquimod (IMQ)-induced mouse model of Psoriasis
1Department of Medicine-Section General Pathology, University of Verona, Verona, Italy, 2University of California, San Francisco, California, United States, 3Department of Medicine-Division of Pulmonology, 1st Department of Internal Medicine, University of Verona, Verona, Italy.

Psoriasis is a chronic skin disease associated with deregulated interplays between immune cells and keratinocytes. Neutrophil accumulation in the skin is one of the histological features that characterize psoriasis. However, the role of neutrophils in psoriasis development remains poorly understood. In this study, we utilized the imiquimod (IMQ)-induced mouse model of psoriasis to elucidate the specific contribution of neutrophils to psoriasis development. We report that neutrophils act as negative modulators of disease propagation and exacerbation by inhibiting γδ T cell effector functions via NADPH oxidase-mediated reactive oxygen species (ROS) production, as revealed by analysing disease development in neutrophil-depleted mice. We also report that Syk functions as crucial molecule mediating neutrophil and γδ T cell interactions. In support of the latter findings, we demonstrate that the specific impairment of Syk-dependent signalling in neutrophils only, is sufficient to reproduce the enhancement of skin inflammation and γδ T cell infiltration observed in neutrophil-depleted mice. Overall, our findings add new insights into the specific contribution of neutrophils to disease progression in the IMQ-induced mouse model of psoriasis. Considering that, similarly to mouse psoriasis, the important role of IL-17 producing γδ T cells in human psoriasis has just started to emerge, it is likely that inhibitory crosstalk between neutrophils and γδ T cells may exist also in human psoriasis. Neutrophils may indeed act as unexpected negative players of disease development in specific types or clinical stages of human psoriasis. Consequently, also the utilization of therapeutic interventions targeted to inhibit neutrophil functions should be carefully evaluated.

P.C6.04.03
Innate-like T-lymphocyte deficiencies in the pathogenesis of chronic obstructive pulmonary disease
P. Engelmann, K. Boddi, N. Farkas, V. Sarosi, Z. Baliko, T. Berek, M. Szabo
1Department of Immunology and Biotechnology, Clinical Center, Medical School, University of Pécs, Pécs, Hungary, 2Department of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary, 3Department of Internal Medicine, 1st Department of Clinical Center, Medical School, University of Pécs, Pécs, Hungary.

Chronic inflammation of the small airways and the damage of lung parenchyma are considered as the major mechanisms in COPD. Recent observations claim that innate-like T-lymphocytes such as invariant natural killer T (iNKT) cells and mucosal-associated invariant T (MAIT) cells connect innate and adaptive immunity. Up to now are scarce data about their involvement in the pathogenesis of COPD. We aimed to observe the proportions of iNKT and MAIT cells in the peripheral blood and sputum samples of stable and exacerbating COPD patients. By means of multicolor flow cytometry the frequencies of total iNKT and MAIT cells and their subpopulations were enumerated. In addition, the expression levels of NKp30, and MAIT TCR genes, along with CD1d, MR1 genes were assessed by qPCR in the study cohorts. Proportions of total iNKT and MAIT cells were dramatically dropped in COPD blood samples. In the sputum of COPD patients reduced numbers of iNKT cells were observed. Furthermore decreased DN and increased CD4+ iNKT subsets, while elevated DN and dropped CD8+ MAIT subpopulations were measured in COPD samples. Reduced invariant TCR mRNA levels in COPD patients had confirmed these findings. CD1d and MR1 mRNA expression were increased in stable and exacerbating COPD patients. In contrast, both molecules were decreased following antibiotic and systemic steroid treatments. Our observations support the notion that iNKT and MAIT cells are involved in COPD. These innate-like T-lymphocytes deserve further analysis to validate their usefulness as potential biomarkers in the pathogenesis of this disease.

P.C6.04.04
The effects of intravenous immunoglobulin (IVig) on peripheral blood polymorphonuclear cells
B. Geçkin, B. Kayaoğlu, M. Gürsel
Department of Biological Sciences, Middle East Technical University, Ankara, Turkey.

IVig has been used in clinical treatment of primary immune deficiencies (PIDs) and several autoimmune diseases. Neutrophil dysregulation frequently observed in a variety of PIDs indicates the importance of neutrophils in the regulation of immune responses. This study aimed to assess the effects of IVIg treatment on neutrophil functions as well as the response of IVIg treated patients to various TLR and/or NLR pattern recognition receptor ligands. IVIg (0.2, 1, 5, 25 mg/ml). Reactive oxygen species (ROS) production was quantified using DHR123 staining and flow cytometry. Similarly, the effect of IVIg treatment on LPS, Zymosan or PMA-induced NADPH oxidase-mediated reactive oxygen species (ROS) production, as revealed by analysing disease development in neutrophil-depleted mice. We also report that Syk functions as crucial molecule mediating neutrophil and γδ T cell interactions. In support of the latter findings, we demonstrate that the specific impairment of Syk-dependent signalling in neutrophils only, is sufficient to reproduce the enhancement of skin inflammation and γδ T cell infiltration observed in neutrophil-depleted mice. Overall, our findings add new insights into the specific contribution of neutrophils to disease progression in the IMQ-induced mouse model of psoriasis. Considering that, similarly to mouse psoriasis, the important role of IL-17 producing γδ T cells in human psoriasis has just started to emerge, it is likely that inhibitory crosstalk between neutrophils and γδ T cells may exist also in human psoriasis. Neutrophils may indeed act as unexpected negative players of disease development in specific types or clinical stages of human psoriasis. Consequently, also the utilization of therapeutic interventions targeted to inhibit neutrophil functions should be carefully evaluated.

P.C6.04.05
Fragile neutrophils in the peripheral blood: a new phenomenon in critically ill patients.
UMC Utrecht, Utrecht, Netherlands.

The purpose of this study was to investigate overall neutrophil function and clinical course of patients who have non-viable neutrophils. Methods: The percentage of PI-positive neutrophils is routinely measured in every blood analysis, as indicator for timely analysis. However, surgical ward and ICU samples are immediately analyzed. Surgical patients who had > 5% PI-positive neutrophils, were included. After inclusion, the percentage of PI-positive neutrophils was reassessed by flow analysis. In addition, phagocytosis was analyzed. Results: Thirty patients were included. The high PI signal originated either from increased neutrophil autofluorescence (n = 7) or the presence of fragile neutrophils (n = 6). Fragile neutrophils were neutrophils that became PI-positive after minimal ex vivo manipulation (red blood cell lysis) or when kept in EDTA tubes. Four of 6 patients with fragile neutrophils died. In contrast, no patients with autoflourescent neutrophils died. Phagocytosis of patients with fragile neutrophils was not impaired. Conclusion: Non-viable neutrophils detected by the hematology analyzer are either true fragile neutrophils or autofluorescent neutrophils. Fragile neutrophils are easily missed during routine blood testing as they are typically lost during standard leukocyte processing. The presence of fragile neutrophils was associated with a high mortality rate and thus renders more attention to determine their role in the clinical course of critically ill patients.

P.C6.04.06
Extracellular neutrophil-derived glycodies - new post-secretional modifiers of human immunoglobulin G glycosylation?
J. Knauf, L. E. Munoz, M. Hermann
Universitätsklinikum Erlangen - Medizin 3, Erlangen, Germany.

Glycosylation of Asparagine™ of Immunoglobulin G (IgG) heavy chain influences the effector functions of IgG and contributes to the pathogenesis of chronic inflammatory autoimmune diseases such as Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA). We employed mass spectrometry to analyze the glycosylation of total IgG from patients with SLE or RA compared to acute inflammatory diseases such as sepsis, as well as healthy controls. Patients with sepsis showed a similar IgG glycosylation pattern as RA patients with low levels of galactose and bisecting N-acetylgalactosaminyl (GalNAc) expression. Surprisingly, the glycosylation pattern of IgG from patients with SLE displayed only minor changes when compared to healthy controls. This is most likely due to the successful treatment and the concomitant low disease activity in our SLE cohort. We tested whether the changes observed for sepsis and RA patients are due to post-secretional modifications by neutrophil extracellular traps (NETs) borne glycodies of IgG glycans. The potential glycan modifiers in muramidase1, beta-galactosidase and beta-N-acetylglucosaminidase have been expressed in human neutrophils and aggregated NEts contained enzyme activities as assessed by specific substrate conversion. Strikingly, co-incubation of IgG with aggregated NETs reduced the percentage of bisecting N-acetylgalactosaminyl (GalNAc). To further analyze the role of glycodies in modifying IgG glycans, we are going to express the specific glycodies and analyze their effect on the glycans of circulating IgG and its functional implications in vivo.

221 words
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.C6.04.07

Implication of type 2 innate lymphoid cells in skin fibrosis during systemic sclerosis

P. Laurence1, M. Jelidi1, P. Manicki1, V. Jolivel2, C. Richer2, P. Ouffoun1, E. Lazaro1, J. Déchanet-Merville1, P. Blanco3, T. Pradeu1, C. Cantin-Bordes1, M. Truchetet1,2

1Immunology laboratory, EA 1833, Paris, France, 2Rheumatology department, CHU Bordeaux Hospital, Bordeaux, France, 3Internal Medicine department, CHU Bordeaux Hospital, Bordeaux, France, 4Immunochemistry department, CHU Bordeaux Hospital, Bordeaux, France.

Systemic sclerosis (SSc) is an autoimmune disease characterized by vascular abnormalities, immune disorders and fibrosis. Recently, some reports highlighted the fundamental role of type 2 innate lymphoid cells (ILC2s) in fibrosis. We hypothesized that ILC2s could be involved in SSc fibrosis, offering new therapeutic perspectives.

Methods: We investigated circulating and cutaneous ILCs in healthy donors and SSc patients by flow cytometry and immunofluorescence. In HCOI-induced SSC mice, we phenotyped ILCs in skin and lung. Results: In 39 SSc patients, circulating ILCs decreased in absolute value (0.019 for HD vs 0.055 for patients, p<0.0001) compared to 18 controls, mainly in ILC2s (0.0138 for HD vs 0.00767 for SSc, p=0.0496) and ILC5s (0.02152 for HD vs 0.003839 for SSc, p<0.0001). We observed an inverse correlation between circulating ILC2s and rodan scores in SSc patients (r=-0.03, p<0.02). A skewing toward ILC2 was also observed in SSc skin patients. HCOI-induced SSc mice showed an early increase in absolute count of ILCs in skin (p=0.02), which was also correlated with collagen content and IL-13 mRNA expression while it remained unchanged in spleen.

Conclusion: We observed a decrease of circulating ILC2s and an increase of ILC5 in SSc skin, correlated with the extent of cutaneous fibrosis. In SSc mice, an increase of skin ILC2 is observed suggesting that these cells could be the trigger of fibrosis in SSc and may constitute a future therapeutic target.

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Toll-like receptor 3 (TLR3) L412F differentially modulates TLR3-9 and non-TLR responses indiopathtic pulmonary fibrosis (IPF) patients: implications for accelerated disease progression

A. N. McElroy1, D. N. O’Dwyer1, A. Tynan1, L. Mawhinney1, P. G. Fallon1, A. G. Bowier2, C. M. Hogaababam3, N. Hiran4, M. E. Armstrong5, S. D. Donnelly1

1School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland, 2School of Medicine and Medical Science, College of Life Sciences, UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland, 3School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland, 4Department of Pathology, University of Michigan Medical School, Michigan, United States, 5Queen’s Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom, 6Department of Clinical Medicine, Trinity Centre for Health Sciences, Tallaght Hospital, Dublin, Ireland.

Introduction: In this study, we investigated the role of the toll-like receptor 3 (TLR3) and TLR2/6 in responses to viral in our human lymphoid fibroblasts from TLR3-9 and non-TLR agonists.

Materials and Methods: Patients with IPF were genotyped for TLR3 L412F and the effects was assessed in primary human lung fibroblasts treated with a panel of TLR3-9 agonists and non-TLR agonists. Cytokine, chemokine and type I interferon levels in IPF fibroblasts were determined by ELISA and qPCR, respectively.

Results: We demonstrated that TLR3 L412F reduced IL-1β production following activation of: TLR3, TLR7, TLR8, TLR7/8, TLR2/6 and in fibroblasts from 412F-heterozygote IPF patients compared with wild-type patients. TLR3 L412F also attenuated TLR3- and TLR4-induced activation of IRF3-dependent IFN-β and Nrf2 activation in 412F-heterozygote IPF patients. Interestingly, 412F-heterozygote IPF fibroblasts also had attenuated responses to the non-TLR ligands, Poly(dA:dT) and PMA.

Conclusions: This study demonstrates that the effects of TLR3 L412F are not limited to TLR3 and that TLR3 L412F can attenuate additional TLR and non-TLR signalling pathways. These findings may have implications for IPF patients during bacterial or viral infection and hence, accelerated disease progression in 412F-heterozygote patients.

P.C6.04.09

Inhibition of peptidyl-arginine deiminases impairs NLRP3 inflammasome assembly and the release of pro-inflammatory IL-1β in macrophages

n. mishra1, F. Ahmed1, L. Ghebremariam1, M. M. Lerch1, R. E. Schmidt2, L. Bassaller2

1Section of Rheumatology, Department of Medicine A, Greifswald, Germany, 2Department of Dermatology, University of Heidelberg, Heidelberg, Germany, 3Department of Medicine A, University Medicine Greifswald, Greifswald, Germany, 4Department of Clinical Immunology and Rheumatology, Hannover Medical School, Hannover, Germany.

Inflammasomes are cytosolic pattern recognition receptors of the innate immune system. They assemble into multi-protein complexes secondary to the recognition of pathogenic stimuli as well as host derived molecules. The macromolecular aggregation of the receptor protein Nod like receptor (NLRs) with the adaptor protein ASC and the effector protein procaspase 1 through homotypic domain interaction into ‘ASC specks’ controls the release of proinflammatory cytokine IL-1β. The host derived molecules, high intracellular calcium concentrations or calcium mobilization from endoplasmic reticulum stores triggers NLRP3 inflammasome activation in macrophages. Peptidyl-arginine deiminase (PAD) are class of calcium dependent enzymes that catalyze the post-translational modification of arginine residues into citrulline amino acid. Of the five known PAD isoforms, only PAD2 and PAD4 are expressed in macrophages.

High intracellular calcium concentration is essential for PAD activity that can be reached in dying cells. The role of intracellular calcium best shown by intracellular Ca2+-dependent PAD enzyme activation. We report here that protein citrullination is common following NLRP3 inflammasome activation in murine macrophages. Interestingly, ASC specks are citrullinated as well. Furthermore, pad enzyme inhibition resulted in a dose-dependent reduction in the release of active caspase-1 and IL-1β in macrophages. Consistently, we observed a reduction in ASC speck formation following pad inhibition. Genetic deficiency of Pad 4 alone was not sufficient to block IL-1β release. However, siRNA knockdown of PAD 2 within PAD 4/- macrophages blocked IL-1β release. In conclusion, we find that PAD enzymes fulfill a previously unknown role in NLRP3 inflammasome assembly and IL-1β maturation.

P.C6.04.10

Polysaturated omega-3 fatty acids have immunosuppressing effects on NK cell phenotype in vitro

S. Y. Omarsdottir1*, K. N. Jensen1, J. Freyssott1, J. Hardardottir1

1National University Hospital of Iceland, Reykjavik, Iceland, 2University of Iceland, Reykjavik, Iceland.

Dietary fish oil, rich in omega-3 polysaturated fatty acids (n-3 PUFA), enhanced resolution of antigen-induced inflammation in mice and induced an early increase in the number of NK cells at the inflamed site, indicating that NK cells may play a role in the resolution of inflammation. The objective of this study was to examine the effects of n-3 PUFA on NK cells in vitro. NK cells were isolated from buffy coat using MACS negative selection. They were cultured for 18 hours with/without 50 μM of the omega-3 PUFA EPA or DHA, or with the omega-6 PUFA arachidonic acid (AA), and then stimulated with IL-12, IL-12 and IL-15 for 24 hours. Cytokine concentration in the medium was measured by ELISA and expression of surface markers by flow cytometry. NK cells cultured with DHA secreted less TNFα (14%), CCL3 (17%) and CCL20 (44.5%) than NK cells cultured with fatty acids. NK cells cultured with DHA also had lower mean expression of the surface markers CXCR1 (14%), NKG2A (6.5%) and CXCR3 (5%). NK cells cultured with EPA secreted less TNFα (13.5%) but more IFNγ (12%) than NK cells cultured without fatty acids. DHA decreased NK cell secretion of neutrophil recruiting cytokines and chemokines (TNFα-ca, CCL3 and CCL20) and decreased NK cell expression of chemokine receptors (CXCR1 and CXCR3) that mediate migration of immune cells to the inflammasome site. These results indicate that n-3 PUFA may modulate NK cell activity and thereby affect inflammation and its resolution.

P.C6.04.11

The cytokytic function of NK cells in the central nervous system is regulated by CD69

M. Relaño Oroz1, A. Tusingina1, C. Torroja1, M. J. Gómez1, A. Angulo1, C. Mulero1, C. Schwäizer1, M. Toulson1, P. Martini1

1Spanish National Center for Cardiovascular Research, Madrid, Spain, 2Faculty of Medicine, Barcelona University, Barcelona, Spain, 3La Princesa Hospital, Madrid, Spain, 4Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

Introduction: CD69 is a C-type lectin family receptor that is expressed upon activation and exerts anti-inflammatory effects. Previous works have shown that CD69-/- mice are resistant to tumour growth and vaccinia virus infection in part due to NK cells activity. However, the specific contribution of these cells and the mechanism by which CD69 could be regulating its function has not been described. The activation of NK cells depends on the balance between activating/inhibitory signals. Thus, in this work we study the membrane receptor repertoire of CD69-/- NK cells using a multiparametric approach in vivo and in vitro.

Materials and methods: We used the new mass cytometry (CyTOF) technology to perform an unbiased multiparametric phenotyping of CD69-/- NK cells and using different animal models we studied the in vivo consequences of this phenotype in contexts such as anti-viral and anti-tumour immunity and GvHD resistance (allogenic cells recognition).

Reults: Our multiparametric study revealed that CD69-/- NK cells present an altered inhibitory/activating receptor repertoire with higher levels of non-self and missing self recognition receptors. This makes them more efficient in the killing of allogenic and tumour cells, being CD69-/- mice resistant to alloGVH. All these effects can be reproduced by an anti-CD69 mAb treatment.

Conclusions: CD69-/- NK cells are more efficient in the killing of tumour and allogenic cells due to different expression of activating/inhibitory receptors. This study suggests that anti-CD69mAb treatment could be employed in the clinics to reduce GVH effects potentially maintaining GVH effects.
Circulating extracellular vesicles from patients with granulomatosis with polyangiitis stimulate neutrophils to generate reactive oxygen species and neutrophil extracellular traps


1Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland; 2Institute of Pharmacology, University of Bern, Bern, Switzerland; 3Department of Clinical Immunology, Jagiellonian University Medical College, Krakow, Poland.

Introduction Activation of neutrophils is one of the key mechanisms observed in pathology of granulomatosis with polyangiitis (GPA). In this study we evaluate if extracellular vesicles circulating in plasma of GPA patients can contribute to this process. Material and methods Extracellular vesicles (EV) from plasma of GPA patients in active stage of the disease (n=10) and healthy controls (n=10) were isolated by ultracentrifugation and characterized by flow cytometry (CD9, CD63, CD81 expression) or nanoparticle tracking analysis. Targeted oxylipins lipodromics of EV was performed by LC-MS. Neutrophil extracellular traps formation (NETs) by neutrophils stimulated with EV or oxylipins was analyzed by fluorescent microscopy or Pico Green assay. ROS production and neutrophils EV binding/uptake were evaluated by flow cytometry. Results EV isolated from plasma of GPA patients stimulated neutrophils to produce reactive oxygen species and release of mitochondrial DNA. However this was observed only when neutrophils were primed with GM-CSF. Priming with GM-CSF increased neutrophils EV binding/uptake as well. Extracellular vesicles isolated from plasma of GPA patients had higher concentration of leukotriene B4 (LTB4) and 5-oxo-eicosatetraenoic acid (5-oxo-ETE) comparing to EV from healthy controls. Moreover, neutrophils stimulated with these oxylipins (LTB4 or 5-oxo-ETE) responded in ROS and NET production in a concentration dependent manner. Conclusions Presented results reveal the potential of extracellular vesicles to activate neutrophils. ROS production and NET formation by stimulated neutrophils is probably linked to EV oxylipins cargo. The study was supported by National Center of Science in Poland, grant number: 2016/21/D/ N26/02123.

The presentation and characterization of Mucosal Associated Invariant T-cells in renal tissue

M. L. Terpstra1, M. J. Singmei2, E. Remmerswaal2, M. C. Van Alderen2, J. Kers2, S. E. Geerlings2, F. J. Bemelman2

1Renal Transplant Unit, Division of Nephrology, Department of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands; 2Department of Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands, 3Department of Pathology, Academic Medical Center, Amsterdam, Netherlands, 4University of Amsterdam, Van ’t Hoff Institute for Molecular Sciences (HIMS), Amsterdam, Netherlands; 5Division of Infectious diseases, Department of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands.

Introduction Mucosal Associated Invariant T (MAIT) cells are innate-like T-cells in the antibacterial and fungal response by recognizing riboflavin metabolites produced by these organisms. MAIT cells are present in human blood, and are highly abundant in the mucoza of the liver, lungs and intestines. It is unclear whether MAIT cells are present in renal tissue and how they phenotypically compare to circulating MAIT cells.

Methods We used a fluorescence-labeled M1-tetramer in conjunction with 14-color flowcytometry to identify and characterize MAIT cells in healthy tissue collected from kidneys surgically removed because of renal cell carcinoma (adjacent non-tumorous tissue) (n=5), in renal allografts explanted after allograft failure (n=14) and in blood from healthy donors (n=5).

Results The mean percentage of MAIT cells within the lymphphocyte was 0.11% (control kidneys) vs 1.10% (renal allografts) and 0.93% (blood) (p=0.05). Due to MAIT cell counts <25 (predefined cutoff value) characterization of MAIT cells was impossible in the control kidneys. MAIT cells in renal allografts appeared to have a relative cytotoxic, activated and tissue resident profile compared to the healthy blood samples, with a significantly higher expression of granzyme B, Ki-67 and CD63/CD103 (p<0.01) and a lower expression of CD95, CD127, CD161 and KLRG1 (p<0.01).

Conclusion MAIT cells can be detected in renal tissue. Though non-significant, MAIT cell percentages seemed to be higher in renal allografts than in healthy renal tissue. MAIT cells in renal allografts consist of a distinct population with a different expression profile than MAIT cells that are present in healthy blood.

Altered phenotype and cytotoxic potential of NK cells in patients with systemic juvenile idiopathic arthritis and macrophage activation syndrome

J. Vanden haute1, M. Imbrechts1, E. Dutreerts1, L. De Somer1, O. Wouters1, R. Mattheys1

1Rega Institute for Medical Research, KULeuven, Leuven, Belgium; 2Laboratory of Experimental Transplantation, KULeuven, Leuven, Belgium; 3Pediatric Rheumatology, University Hospitals Leuven, Leuven, Belgium.

Introduction Systemic juvenile idiopathic arthritis (sJIA) is a severe immunoinflammatory childhood disease characterized by arthritis and systemic features. About 10% of sJIA patients develop macrophage activation syndrome (MAS), a life-threatening hyperinflammatory cytokine storm syndrome characterized by excessive activation of T cells and macrophages.

Objective sJIA and MAS are linked to defects in cytotoxicity of NK cells. In this study we investigated the phenotype and cytotoxic potential of NK cells in sJIA and MAS patients. Results Extensive phenotypic analysis of blood cells from active and inactive sJIA and active MAS patients revealed a decreased expression of cytotoxic proteins (i.e. perforin and granzyme) and K+ transmembrane expression in the expression of inhibitory and activating receptors, with decreased expression of CD57 and Killer Immunoglobulin Receptors (KIR) and increased levels of NKp44 by NK cells. In addition, the activation status and expression of ligands for NK cell receptors was analyzed on monocytes of sJIA and MAS patients. To investigate the cytotoxic potential of NK cells against autologous activated monocytes we optimized a protocol, which allows us to compare NK-specific cytotoxicity against K562 tumor target cells and autologous activated monocytes from patients and healthy controls. sJIA patients showed a defective IL-18-induced IFN-γ expression in NK cells, which is absent in MAS patients and partially resolved in inactive sJIA patients. Conclusion NK cells of sJIA and MAS patients show an altered phenotype. Subtle defects in their IFN-γ production and autoligous killing of activated immune cells may underlie the immune-inflammatory dysregulation in sJIA. Results funded by FWO.
It was shown that the predominant majority of married couples were identified by the HLA-DRB1 gene, which was more often associated with herpervirus infections. Despite the fact that the level of PCR-positive results in the group of married couples with primary infertility was higher than in married couples with secondary fertility (100 and 92%, respectively), the maximum values of titres of specific antibodies to herperviruses, including the detection rate of markers Exacerbations of infection (IgM antibodies) in the group of married couples with secondary sterility were higher than in the group of married couples with primary infertility, and significantly higher than in married couples with normal fertility. Therefore, it is this histocompatibility antigen - HLA-DRB1 - that is associated with a significant frequency of sterility and infectious contamination with herperviruses (HSV 1, 2 types, CMV, VEB) in married couples with secondary infertility. The marker HLA-DRB1, most often found in married couples with infertility, including idiopathic, is associated in this contingent not only with significant contamination with herperviruses, but also with the presence of a chronic infectioius process in the reaction stage. Conducted clinical and immunological studies clearly demonstrate the need for a comprehensive examination of women with functional disorders in the immunogenesis system with mandatory assessment of the level of infectious (virus) contamination and allows us to propose a comprehensive examination algorithm for functional disorders in the immunogenesis system, including the study of the level of the viral load as a necessary component. This publication was prepared with the support of the “RUDN University Program 5-100”.

**P.C6.04.19** Evidence of the NLRP3 Inflammasome-Complement Axis in Osteoarthritis

J. Bramhall, C. Bessant, J. Neisen, I. Osuch, A. Dawson, K. Triantafiliou, E. Nicholas; GlaxoSmithKline, Stevenage, United Kingdom.

Osteoarthritis (OA) has long been considered a “wear and tear” disease, but recent findings suggest that chronic low-grade inflammation plays a role in its pathogenesis. The synovial tissue has also taken center-stage as target and producer of inflammatory stimuli. Evidence from patient and rodent studies strongly implicates complement in OA pathogenesis. However, the downstream mechanisms have not been elucidated. An improved mechanistic understanding will enable design of therapeutic strategies. This study aimed to explore how complement may mediate synovial inflammation.

Analysis of patient tissues (synovial fluid, synovium) confirmed complement activation in OA. Membrane attack complex (MAC) deposition and increased IL-1β suggest involvement of the NLRP3 inflammasome. This led to the hypothesis that chronic inflammation in OA may depend on a synergistic complement-inflammasome interaction.

To validate the patient data, we examined MAC-mediated inflammasome activation in cell lines and primary synovium. Sub-lytic MAC challenge of the synovial membrane biopsies and synovial cell line resulted in an IL-1β response, suggesting a complement-inflammasome interaction. This response was dependent on both CS, NLRP3 and PI3K. Our data suggests that CSa provides a priming signal for inflammasome activation. MAC was found to provide signal 2, but is also able to trigger inflammasome activation independently of CSa.

In conclusion, terminal pathway effectors can trigger both signals of inflammasome activation, providing a mechanism in which the terminal pathway of complement may perpetuate inflammation in OA.

(Human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.)

**P.C6.04.20** StemCell therapy stabilizes atherosclerotic plaques after myocardial infarction

W. Woudstra1,2,3, A. van Broekhoven1,2,3, E. Mees1,2, M. Koogman1, M. C. Marrison1, A. C. van Rossum1, M. N. Holder2, L. J. Juffermans2,3, H. W. Niessen1,2,3, P. A. Krijnen1,2,3

1Department of Pathology, VU University Medical Center, Amsterdam, Netherlands, 2Amsterdam Cardiovascular Sciences, Amsterdam, Netherlands, 3Department of Cardiothoracic Surgery, VU University Medical Center, Amsterdam, Netherlands, 4Department of Metabolic and Cardiac Health Research, The Netherlands Organization for Applied Scientific Research (TNO), Leiden, Netherlands, 5Department of Cardiology, VU University Medical Centre, Amsterdam, Netherlands, 6Department of Oral & Maxillofacial Surgery, VU University Medical Centre, Amsterdam, Netherlands.

Background: Myocardial infarction (MI) accelerates atherosclerosis through increased plaque inflammation and destabilization resulting in an increased risk of recurrent MI. Mesenchymal stem cells (MSC) are a promising therapeutic option for atherosclerosis. Here we investigated the effect of StemCell therapy (MSC-microbubble complexes combined with ultrasound) on atherosclerotic plaques after MI.

Methods: MI was induced in atherosclerotic ApoE-/- mice. Six days post-MI, intravenous StemBells or vehicle were given, followed by 1 minute of transthoracic ultrasound. The effects of StemCell treatment on plaque size and stability and the infarcted heart were determined 28 days post-MI. Moreover, monocyte subtypes and lipids in the blood were studied.

Results: StemCell treatment significantly increased cap thickness, decreased intra-plaque macrophage density and increased the percentage of intraplaque anti-inflammatory macrophages. Plaque size and serum cholesterol and triglycerides were not affected. Furthermore, StemCell treatment significantly increased the percentage of anti-inflammatory macrophages within the infarcted myocardium, but did not affect cardiac function nor infarct size. Finally, the percentage of anti-inflammatory monocytes in the circulation was increased after StemCell therapy.

Conclusion: Systemic StemCell therapy decreased plaque inflammation and destabilization, predominantly associated with local and systemic effects on macrophages/monocytes. Hence, StemCell therapy may be a therapeutic option to prevent atherosclerosis acceleration after MI.

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**P.C6.05.01** Innate control of inflammation and tissue repair - Part 5

**P.C6.05.02** Arginine metabolism during Behçet disease: correlation with the clinical expression

H. Belqasme1, M. L. Ahmed1, A. Chekroud1, K. Lahmari1, D. Hakemi1, Z. Djebali1, F. Otmani1, C. Touil-Boukaffa2

1Cytokines and NO Synthases team, LBCM, USTHB, Algiers, Algeria, 2Internal medicine department, CHU Bab ElOued, Algiers, Algeria, 3Internal medicine department, CHU Mustapha Bacha, Algiers, Algeria.

Behçet disease (BD) is a chronic systemic inflammatory disorder with uncertain etiology. In previous studies, we showed an increase in nitric oxide (NO) production during disease active stages. NO is the product of NO synthases. It is synthesized from L-Arginine. However, this amino-acid is also the substrate of other enzymes. In this study, we investigated the activity of the other enzymes implicated in arginine metabolism. 65 patients fulfilling BD diagnosis criteria were included in the study (35 active and 30 inactive) and 25 healthy controls (HC). The activity of NOS, ARG, ADC, AGAT and GAMT were assessed through their products measurement. IL1β and IL-27 were measured by ELISA. Mann Witney U test was used for statistical comparison. Spearman test was used for correlation study.

Our results showed a significant increase in all enzymes activity during BD in comparison to HC (p<0.05). In addition, we observed a significant increase of NOS and ADC activities and IL-1β during active BD in comparison to inactive BD (p<0.01) or HC (p<0.001). Also, NOS and ADC showed increased activities during inactive BD in comparison to HC (p<0.05). Correlation analyses showed no significant correlations between the different enzymes however a significant positive correlation was observed between NOS and IL-1β (r=0.62, p<0.05) while a negative correlation was observed between IL-27 and arginase (r=-0.45, p<0.02). In conclusion, our results showed an increased activity in arginine metabolism during BD especially NOS and ADC in relation with disease activity. Moreover, this activity seems to be related to cytokines production.

**P.C6.05.03** Gastro protective and anti-inflammatory activities of Pistacia lentiscus L. extracts against gastric ulcer in rats

I. BOUTEMINE1, M. Amiri1, Z. Amiri1, C. Fitting1, J. Cavallion1, C. Touil-Boukaffa2

1University of Sciences and Technology Houari Boumediene, algiers, Algeria, 2Pathology department, Mustapha Pacha Hospital, Algiers, Algeria, 3Cytokines & Inflammation, Pasteur Institute, Paris, France.

Aim of the study: In the present study, we investigated the anti-ulcerogenic and anti-inflammatory activities of fatty oil (PLFO) and aqueous extract (PLAE) of Pistacia lentiscus on ethanol-induced gastric ulcers in Wistar rats. Material and methods: PLFO and PLAE were orally administered to rats before gastric ulcer induction by ethanol. The lesions of the gastric mucosa were evaluated by macroscopic and histopathological examination. In addition, the amount of nitric oxide (NO) and pro-inflammatory cytokines (tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)) were assessed in the plasma and the supernatant of explants cultures of gastric mucosa. Finally, the mucus production and INOS (inducible NO synthase) expression were determined by histochemical and immunohistochemical analysis respectively. Result: Our results indicated that the pretreatment with PLFO and PLAE significantly reduced the areas of gastric ulceration and hemorrhage. Interestingly, pretreatment with these extracts highly reduced the plasmatic concentration of NO.
In addition, a significant decrease of NO, IL-6 and TNF-α levels was observed in explants culture. Moreover, IND0 expression was also reduced in the gastric mucosa. In contrast, miRNA-147 overexpression in gastric mucosa cells was enhanced. Interestingly, the histopathological analysis of the gastric mucosa has indicated that PLFO and PLAE pretreated groups displayed normal histology. Conclusion: Our results demonstrate that PL extracts display significant pro-inflammatory effects against gastric ulcer. Importantly, the mechanism underlying PLFO and PLAE normal activities might implicate the inhibition of inflammatory responses during gastric ulcer.

P.C.06.05.04 Engagement of Fas differentially regulates the production of LPS-induced pro-inflammatory cytokines and type I interferons
C. Lyons1, K. Brennan1, S. Doyle1, A. Houston1, E. Brit1

1University College Cork, Cork, Ireland, 2Trinity College Dublin, Dublin, Ireland, 3Trinity College Dublin Dublin, Dublin, Ireland.

Best known for its role in apoptosis, recent reports suggest that Fas (CD95) signalling is also involved in other cellular responses including inflammation. Whilst Fas and its adaptor protein FADD have been previously shown to negatively regulate LPS-induced pro-inflammatory responses, their role in LPS-induced type I Interferon production is unknown. Here we show that Fas engagement on TH-1 macrophages using an agonistic Fas antibody CH11, augments LPS-induced NF-κB responses, causing an increase in the production of TNFα, IL-10, IL-8, IL-6 and IL-12. Conversely, co-stimulation with both LPS and CH11 causes a significant reduction in the level of IFNβ production. This differential effect involves the Fas adaptor FADD as, whilst LPS-induced IL-6 production was increased in FADD−/− murine embryonic fibroblasts, LPS-induced IFNB production was significantly reduced in these cells. Overexpression of a dominant negative form of FADD, the FADD-Death Domain (FADD-DD), in the RAW264.7 macrophage cell line, inhibits LPS-induced IFNβ-luciferase but not LPS-induced NF-κB-luciferase. In contrast, overexpression of full-length inhibited LPS-induced NF-κB-luciferase activation but was seen to augment LPS-induced IFNB-luciferase. Moreover, the FADD-DD inhibits TRIF- and TRAM-induced IFNB-luciferase production, indicating that FADD may be interacting with the TRL-4 pathway at the level of these TR4a adaptor proteins. In conclusion, these data identify FADD as a novel component of the MyD88-independent pathway leading from TR4 to Type I Interferon production and moreover demonstrate that both Fas and its adaptor FADD can differentially regulate the production of LPS-induced pro-inflammatory cytokines and type I Interferons

P.C.06.05.05 mirRNA-147 targets the electron transport chain component NDUFA4 in response to inflammatory stimuli
S. A. Clayton1, S. W. Jones1, M. Kurwasa-Stolarska4, A. R. Clark1

1University of Birmingham, Birmingham, United Kingdom, 2Arthritis Research UK Rheumatoid Arthritis Pathogenesis Centre of Excellence (RACE), Glasgow, Birmingham, Newcastle, United Kingdom, 3University of Glasgow, Glasgow, United Kingdom.

Introduction: miRNAs are an important class of post-transcriptional regulator, with many being described as instrumental in immune cell inflammatory responses. miRNAs can alter cellular metabolism by targeting essential components of specific metabolic pathways. Macrophase phenotype and function is now known to be intimately linked to cellular metabolic status, and consequently miRNAs have the potential to achieve immunomodulatory effects by targeting macrophage metabolic pathways.

Methods: MiRNA and miRNA expression was measured by RT-qPCR in primary human monocyte derived and mouse bone marrow derived macrophages in response to pro-/anti-inflammatory stimuli. Several miRNA target prediction tools were used (TargetScan, miranda, miRDB, miRTarBase). NDUFA4 protein expression was detected by Western blotting. Cells were transfected with miRNA mimics (Dharmacon) to validate targets.

Results: Expression of microRNA-147 in macrophages was induced by pro-inflammatory stimuli such as LPS. LPS-induced miR-147 expression was inhibited by the synthetic glucocorticoid dexamethasone in mouse macrophages by up to 93%, and the human homologue mir-147b by up to 81%. Bioinformatic analysis found that mir-147(b) was predicted to target the 3’UTR of electron transport chain component NDUFA4. Expression of human NDUFA4 mRNA and protein were both significantly downregulated by LPS treatment of macrophages, and NDUFA4 protein expression was rescued by co-treatment with dexamethasone. This is consistent with the regulation of mir-17b by these stimuli. Ectopic expression of mir-147b confirmed that this miRNA functionally targets NDUFA4.

Conclusions: Mir-147 has the potential to regulate macrophage metabolism in response to pro-inflammatory signals by downregulating a key electron transport chain component. Arthritis Research UK grant 20289

P.C.06.05.06 ERAP1 depletion mimics the effects of the Behçet’s Disease associated enzyme variant on the HLA-B*51 peptide
P. Guasp3, M. Campagnone4, E. Barone5, J. J. Kuiper5, D. Fruci1, A. Admon6, J. Lopez de Castro2

1Centro de Biologia Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain, 2Immuno-Oncology Laboratory, Pediatric Haematology/Oncoology Department, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy, 3Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel, 4Department of Ophthalmology, University Medical Center Utrecht, Utrecht, Netherlands.

The Endoplasmic Reticulum Aminopeptidase ERAP1 trims peptides to the correct length for presentation by HLA class I molecules. HLA-B*51 shows a high preference for peptides that carry Proline or Alanine at position 2. The Pro2 subpeptide shows higher affinity for HLA-B*51 than the Ala2 subpeptide. ERAP1 was knocked out in the transfectant cell line 721.221-HLA-B*51 using the CRISPR Cas9 technology. The B*51 bound peptidome presented by the WT and ERAP1 KO cells was comparatively analyzed by quantitative mass spectrometry, and the surface expression of HLA-B*51 was measured by flow cytometry. The theoretical affinity of the identified peptides for HLA-B*51 was calculated using predictive algorithms. The ERAP1 depletion dramatically altered the ratio between the Ala2 and Pro2 subpeptides. This effect dramatically reduced the affinity of the peptide presented by the ERAP1 KO cells due to the overrepresentation of the low affinity subpeptide of Ala2 ligands. ERAP1 KO did alter neither the surface expression of HLA-B*51 nor the levels of Free Heavy Chain in this transfectant cell line. Overall, we demonstrate that ERAP1 is required to maintain the balance between Ala2 and Pro2 ligands in HLA-B*51 cells, and its absence leads to a peptide of much lower affinity. The effects of ERAP1 depletion on the HLA-B*51 peptide repertoire are very similar to those observed in the presence of the low activity and Behçet’s associated ERAP1 Hap30 variant. Being the interaction between HLA-B*51 and KIR3DL1 sensitive to the HLA-B*51 peptidome, it is very likely that ERAP1 depletion may affect the NK cell reactivity.

P.C.06.05.07 IgA versus IgG: the potential and danger of IgA as potent immune cell activator
M. H. Heineke;
Vu medical centre, Amsterdam, Netherlands.

Antibody-opsonized pathogens can activate immune cells via Fc receptors. Both IgA Fc receptor (FcαRI) and IgG Fc receptor IIA (FcγRIIA) are thought to initiate similar signaling pathways and responses, but we previously showed that only IgA triggering of neutrophils led to leukotriene B4 release with concomitant neutrophil migration. In this study we investigated cellular activation through IgA or IgG in more detail using different methods, including live cell imaging, (phospho)proteomics and metabolomics. No differences were observed in uptake of IgG- or IgA-coated beads and subsequent release of reactive oxygen species and neutrophil extracellular traps. However, crosslinking of FcαRI led to a slower but stronger and more sustained signaling profile, exemplified by increased intracellular calcium and phosphotyrosine levels. Only IgA stimulation induced downstream events, like release of reactive oxygen species and pro-inflammatory cytokines. Importantly, enhanced activation through FcαRI is not neutrophil specific, as stimulation of monocytes with IgA also led to increased activation. These results support 1) that signaling routes of FcγRIIA differ from those that are initiated by FcαRI, resulting in distinct functional profiles, and 2) IgA is a more potent immune cell activator than previously anticipated. This may have significant implications in autoimmunity, as IgA auto-antibodies are found in a multitude of autoimmune diseases. Moreover, IgA may represent a potent novel immune activator during cancer immunotherapy.

P.C.06.05.08 Lunasin regulates obesity-related inflammation in C57BL/6 mice fed with high fat diet
C. C. Hsieh1, M. J. Chou2, S. H. Peng2

1School of Life Science, Programs of Nutrition Science, Nutrition Science, National Taiwan Normal University, Taipei, Taiwan, 2Department of Human Development and Family Studies (Nutritional Science & Education), National Taiwan Normal University, Taipei, Taiwan.

Accumulating evidence has shown that extra adiposity influences the progression of various chronic diseases. The process of adiposity induces the infiltration of macrophages, accompanied by multiple inflammatory mediators. Lunasin, a natural small peptide, exhibits several biological activities, such as anti-carcinogenesis, anti-inflammatory, and antioxidative activities. The aim of this study is to investigate whether lunasin regulates obesity-related inflammation in mice fed with high fat diet. C57BL/6J mice were fed a low fat (LF), or high fat (HF) diet, and HF intraperitoneal injected by lunasin 4 mg/kg bw (HF-LI) and 20 mg/kg bw (HF-HL) for 7 weeks. Our results showed that the HF mice have significant higher body weight and organs weight including spleen, liver, and adipose tissue than the LF mice. The oxidized lipid malondialdehyde value was significantly higher in the HF group. The mice received lunasin significantly decreased malondialdehyde values in the adipose and liver. Interleukin (IL)-1β production by LPS-stimulated peritoneal cells was increased in HF mice, and were decreased in mice received lunasin treatment.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 423
Histological analysis of the epididymal adipose tissue of HF groups with lunasin treatment showed less F4/80+ macrophage infiltration, especially lower CD11c+M1 phenotype, compared to HF and LF groups. More related mediators should be analyzed in the future. In summary, lunasin regulates obesity related mediators in C57BL/6 mice fed with high fat diet, suggesting the intake of lunasin from diet or a supplement, for auxiliary prevention or therapy in obesity-related application. The authors acknowledge the funding from Ministry of Science and Technology, Taiwan (MOST 106-2311-B-003-005-MY3).

P.C6.05.09
Frequency and activation status of myeloid cells in the Guillain-Barre syndrome
W. van Rijjs, W. Fokkind, A. Tio-Gillen, M. Brem, B. Jacobs, R. Huizinga; Erasmus MC, University Medical Center, Rotterdam, Netherlands.

The Guillain-Barre syndrome (GBS) is an acute immune-mediated neuropathy, which may develop after relatively common infections, including Campylobacter jejuni and cytomegalovirus. Macrophages are important in the pathogenesis of GBS by phagocytosing myelin and axons in the nerve. However, little is known about their precursors in the peripheral blood. Here we assessed the composition and phenotype of monocytes and dendritic cell (DC) subsets in peripheral blood of GBS patients. Peripheral blood mononuclear cells (PBMC) were isolated from GBS patients (n=20), before and after immunomodulatory treatment, and age and gender-matched, healthy controls (n=20). The frequency and phenotype of six myeloid cell subsets was determined by 13-color flow cytometry. The frequency of total monocytes, determined as percentage of CD45+ cells, was significantly increased in GBS patients compared to controls. The monocyte population was skewed towards more intermediate (CD14+CD16+; p<0.05) and less non-classical (CD14- CD16+; p<0.01) monocytes. Increased expression of CD40, TLR2 and Siglec-7 on non-classical monocytes suggested an enhanced differentiation towards non-classical monocytes. Immunomodulatory treatment strongly reduced the frequency of non-classical monocytes and all DC populations in CD45+ PBMC, as well as expression of CD40, HLA-DR and TLR4 on classical monocytes. In conclusion, our data identify significant changes in the monocyte compartment in GBS. Further analysis should reveal whether these changes are related to disease severity and treatment response. Supported by the Benson Clinical Research Fellowship (GBS/CIDP Foundation International).

P.C6.05.11
Dietary fish oil enhances the proportion of mature NK cells in murine antigen-induced peritonitis
K. N. Jensen1, J. Freyssottiri, J. Hardardottiri;
1Londisphati - the National University Hospital of Iceland, Reykjavik, Iceland; 2University of Iceland, Reykjavik, Iceland.

Unresolved inflammatory responses may lead to chronic inflammation, which is linked to the pathogenesis of a number of degenerative diseases in Western countries. We have previously shown that omega-3 PUFA increased an early peak in the number of NK cells in antigen-primed p50-/- mice and also enhanced the resolution of the inflammation. Furthermore, depletion of NK cells prevented the timely resolution of inflammation in the same model. The aim of this study was to determine the effects of dietary omega-3 PUFA on the NK cells at the peak of the inflammation. Mice were fed either a control or a fish oil enriched diet, immunized with methylated BSA (mBSA) and mBSA injected into the peritoneum. Peritoneal cells were harvested at the peak of inflammation, sorted and assessed by flow cytometry. As was shown in the previous study, mice fed the fish oil enriched diet had a lower number of neutrophils and a higher number of total NK cells at the peak of inflammation. More specifically, mice fed the fish oil diet had a higher number of mature NK cells but a lower number of immature NK cells in their peritoneum than mice fed the control diet. These results suggest that dietary fish oil may enhance maturation of NK cells and that these mature NK cells may play an important role in the resolution of inflammation either by limiting the infiltration of neutrophils or inducing neutrophil apoptosis in a more effective manner.

P.C6.05.12
DNase I treatment ameliorates mouse experimental colitis
E. Y. H. Lin1, Y. H. Hsu2, C. S. Husei1, H. W. Chang1, H. S. Chang1;
1Department of Life Science, National Taiwan University, Taipei, Taiwan; 2School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan.

Neutrophils are the most abundant white blood cells with potent antimicrobial activities. Neutrophil extracellular trap (NET) is one of the defense mechanisms utilized by neutrophils to control microbial infections. A recent proteome and microscopy analysis of intestinal biopsies of patients with ulcerative colitis indicated an increased neutrophil abundance and aberrant NETs in inflamed colon tissues. However, the impact of NET in colitis has yet been fully illustrated. Here we demonstrated that administration of DNase I, an endonuclease that dissolves the NET structure, suppressed colitis, expressed inflammation, and sorted out by flow cytometry. As was shown in the previous study, mice fed the fish oil enriched diet had a lower number of neutrophils and a higher number of total NK cells at the peak of inflammation. More specifically, mice fed the fish oil diet had a higher number of mature NK cells but a lower number of immature NK cells in their peritoneum than mice fed the control diet. These results suggest that dietary fish oil may enhance maturation of NK cells and that these mature NK cells may play an important role in the resolution of inflammation either by limiting the infiltration of neutrophils or inducing neutrophil apoptosis in a more effective manner.

P.C6.05.13
Identifying the components of the ubiquitin proteasome system that target the p50 subunit of NF-kB for degradation
J. P. Mitchell, R. J. Carmody; Institute of Infection Immunity and Inflammation, Glasgow, United Kingdom.

Introduction: The NF-kB subunit p50 is a critical regulator of inflammation and is a major transcription factor in most cell types. In macrophages, p50 homodimers act as repressors of transcription to limit the expression of pro-inflammatory cytokines and promote the resolution of inflammation, which is essential for avoiding the damaging effects of prolonged inflammation on the host. The stability of p50 homodimers is an important determinant of this repressor function and is controlled by ubiquitin-triggered proteasomal degradation. Despite its importance, little is known about the molecular mechanisms that target p50 for degradation, or the cellular factors that initiate p50 ubiquitination.

Methods: By using a panel of putative ubiquitin E3 ligases for p50 and a library of p50 mutants containing specific lysine to arginine substitutions, we aim to identify the components of the ubiquitin proteasome system (UPS) that target p50 for ubiquitination and degradation.

Results: Initial data has identified a novel role for SOCS1 as an E3 ligase that ubiquitinates p50. We have also identified a lysine residue of p50 that is targeted for ubiquitination for which we have not yet identified the specific E3 ligases responsible.

Conclusions: These findings indicate that p50 ubiquitination is orchestrated by a complex network of UPS factors with significant implications for our understanding of this regulatory mechanism in the control of inflammation.

P.C6.05.14
Metabolic alterations in airway macrophages during pulmonary fibrosis
P. P. Ogger1, R. J. Hewitt2, P. L. Molyneaux1, T. M. Maher1, C. M. Lloyd2, A. J. Byrne2;
1National Heart and Lung Institute, Imperial College London, London, United Kingdom; 2NIHR Respiratory Biomedical Research Unit, Royal Brompton Hospital, London, United Kingdom.

Background: Idiopathic pulmonary fibrosis (IPF) is a fatal disease with limited treatment options. Evidence suggests that IFP is in part due to dysregulated wound-healing orchestrated by macrophages. While metabolic reprogramming of macrophages drives inflammation, its central role in IPF is unknown. We hypothesise that airway macrophage (AM) metabolic reprogramming underlies IFP and that manipulation of AM metabolism represents a potential novel therapeutic strategy for IFP. Methods: First we assessed AM number and phenotype in patient bronchoalveolar lavage (BAL) by flow cytometry. Subsequently AMs were enriched and metabolic functionality was analysed by extracellular flux analysis and PCR array of key metabolic enzymes. To investigate the metabolic phenotype of tissue resident and monocyte recruited AMs in pulmonary fibrosis, we utilised the bleomycin mouse model and measured expression of metabolic genes and mitochondrial function in sorted macrophage populations. Results: IFP AMs showed increased expression of ENO2 and IDH2, while SDHC gene expression is decreased and oxygen consumption rate is similar compared to healthy controls. Upon bleomycin treatment, Siglec-F+ resident macrocyte recruited airway macrophages gradually replaced Siglec-F- tissue resident airway macrophages and show higher gene expression of HK3 and IDH2. Conclusions: Together these data indicate that during pulmonary fibrosis, AMs undergo metabolic alterations. The differential gene expression of IDH2 and SDHC is particularly interesting considering the drives major metabolic pathways. Future work aims to further characterise the metabolic phenotypes of resident and recruited AMs in the lung and to investigate how alterations in AM metabolic intermediates in IPF may contribute to the disease.
**P.C6.05.15**

**Role of TGF-β1 in airway remodelling in small airways in chronic obstructive pulmonary disease**

J. Ehlert, C. Moritz, P. A. G. van Gennip, M. Wesseling, J. J. de Haan

**Introduction:** Small airways in chronic obstructive pulmonary disease (COPD) contribute to the structural remodelling including airway wall thickening and exacerbation of airway hyperreactivity.

**Aim:** The aim of this study is to analyse the role of TGF-β1 in the remodeling of small airways in COPD.

**Methods:** Adult male Wistar rats with COPD were treated with either bleomycin (7 IU/kg) or sham. Bronchioles of Group I (bleomycin) were compared to Group II (sham) at baseline and 28 days after instillation. TGF-β1 was measured in bronchoalveolar lavage (BAL) fluid using ELISA and western blot analysis.

**Results:** TGF-β1 expression was significantly higher in the bleomycin group compared to the control group at 28 days post-instillation. The increased expression of TGF-β1 correlated with increased collagen deposition and airway wall thickening.

**Conclusion:** TGF-β1 plays a significant role in the remodeling of small airways in COPD, contributing to the structural changes observed in the disease.

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**P.C6.05.16**

**Differentiated monocyte-derived macrophages reduce neutrophil persistence in lung inflammation**

S. Perniola, L. Dinoia, N. Lacapra, D. Natutzu, R. Rizzo, F. Iannone

**Rheumatology Unit - Department of Emergency Medicine and Transplantation, Bari, Italy.**

Background: Resolution process downregulates the inflammation and promotes tissue repair by the Specialized Pro-Resolving Mediators (SPMs), that act by interacting with specific cellular receptors: CMKLRI, BLT1, FR2 and GPR32. In rheumatoid arthritis (RA) the reactive inflammation becomes persistent and the innate immune response turns into the adaptive immune activation.

Objectives: nowadays is recognized that SPMs are involved in RA pathogenesis so we evaluated the expression of CMKLRI, FR2 and BLT1 in RA patients.

Methods: Patients affected with RA were enrolled in this study. At entry, ESR, CRP, DAS28-ESR, CDAI and HAQ were collected. Patients were divided into high-moderate (H-Mo/RA if DAS28-ESR ≥ 3.2) and low remission (L-R/RA if DAS28 < 3.2) disease activity. The expression of CMKLRI, FR2 and BLT1 in peripheral T cells (CD3+) and monocytes (CD14+) was evaluated by flow-cytometry assay.

Results: In RA patients pathogenesis was studied. Similar kinetics of expression were observed on CD14+. L-R/RA vs H-Mo/RA were significantly higher in L-R/RA than in H-Mo/RA (p < 0.0001).

Conclusions: FR2 and CD14+ cells are significantly higher in L-R/RA than in H-Mo/RA (p < 0.01). We demonstrated an inverse correlation between BLT1 monocyte and ESR (p < 0.01), CRP levels (p < 0.008), DAS28-ESR (p < 0.03), CDAI (p < 0.0076) and HAQ (p < 0.0138) and a weak correlation between FPR2 expression and HAQ (p < 0.05).

**P.C6.05.17**

**Surfactant protein D regulates cigarette smoke-induced lung inflammation through inhibition of ceramide synthesis**


**Introduction:** Surfactant protein D (SP-D) is a pulmonary collectin with established anti-inflammatory functions. SP-D-deficient mice exhibit pulmonary emphysema together with elevated inflammation, pro-inflammatory cytokine and chemokine production leading to recruitment of neutrophils into the alveoli to tackle pathogens. During resolution of inflammation, Resident, sessile airway macrophages clear infiltrating neutrophils before monocyte arrival with a minimal neutrophil lymphatic entry.

**Aim:** We hypothesized that surfactant protein D protects mice from pulmonary inflammation and emphysema generation and that SP-D-based therapy might be a future potential treatment of COPD.

**Method:** Wistar rats were euthanized on day 7, 14, 28 after bleomycin/saline instillation: Group I (control, n=18), Group II (bleomycin, 7 IU/kg, n=18), Group III (bleomycin+surfactant, 100mg/kg/d, n=18). Let-7d, miR-21, bFGF, TGF-β1 mRNA and protein levels were assessed. Results: Bleomycin instillation caused upregulation of mir-21 and downregulation of Let-7d from day 7. An associated upregulation of TGF-β1 and bFGF mRNA and protein levels was seen. BFGF increased in AECs, peribronchiolar fibroblasts and reduced in endothelial cells from day 7. Progressive perivascular inflammation, vasoconstriction and vascular smooth muscle cell hypertrophy (VSMCH) was seen. After bosentan therapy, mir-21 downregulation and Let-7d upregulation was seen on day 28. This correlated with a reduction in TGF-β1 and bFGF mRNA and protein levels, reduction in perivascular inflammation and remodeling while vasoconstriction persisted in 28 days, after bosentan therapy. Conclusion: The mir-21-TGF-β1-bFGF-Let-7d axis plays a key regulatory role in the pathogenesis of PAH. TGF-β1 has an autoregulatory feedback loop with mir-21 and bFGF. Loss of endothelial bFGF downregulates Let-7d, increases TGF-β1 and promotes endothelium-mesenchymal transition, leading to VSMCH and vascular remodeling. Bosentan therapy, downregulates mir-21, reduces TGF-β1 signaling, upregulates let 7d and attenuates VSMCH and pulmonary hypertension.

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**P.C6.05.18**

**miR-21-5p expression in chronic obstructive pulmonary disease**

S. J. Lee, J. Kim, S. Kwon, S. C. de Jager, J. P. Sluijter, J. Vestbo

**Introduction:** miR-21 is a robustly upregulated miRNA in human and rodent pulmonary tissues in a wide range of lung diseases. miR-21 plays an important role in developmental processes and, upon misregulation, contributes to sustain chronic inflammation in active RA.

**Aim:** To investigate the expression and function of miR-21 in human and rodent lung tissues from patients with active RA.

**Methods:** Patients with active RA were divided into high-moderate (H-Mo/RA if ESR ≥ 3.2) and low remission (L-R/RA if ESR < 3.2) disease activity. The expression of miR-21 in peripheral T cells (CD3+) and monocytes (CD14+) was evaluated by flow-cytometry assay.

**Results:** In RA patients the pathogenesis was studied. miR-21 was significantly higher in L-R/RA than in H-Mo/RA (p < 0.0001).

**Conclusion:** Our results indicate that miR-21 protects from chronic inflammatory lung inflammation and emphysema generation and that miR-21-based therapy might be a future potential treatment of COPD.

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**P.C6.05.19**

**miR-21 and bovine growth differentiation factor 15 (GDF15) in human and rat lungs**

V. Vallabhbhai, G. Pasterkamp, J. P. Sluijter, J. Vestbo

**Introduction:** miR-21 is a robustly upregulated miRNA in human and rodent pulmonary tissues in a wide range of lung diseases. miR-21 plays an important role in developmental processes and, upon misregulation, contributes to sustain chronic inflammation in active RA.

**Aim:** To investigate the expression and function of miR-21 in human and rodent lung tissues from patients with active RA.

**Methods:** Patients with active RA were divided into high-moderate (H-Mo/RA if ESR ≥ 3.2) and low remission (L-R/RA if ESR < 3.2) disease activity. The expression of miR-21 in peripheral T cells (CD3+) and monocytes (CD14+) was evaluated by flow-cytometry assay.

**Results:** In RA patients the pathogenesis was studied. miR-21 was significantly higher in L-R/RA than in H-Mo/RA (p < 0.0001).

**Conclusion:** miR-21 may be a potential therapeutic target in chronic obstructive pulmonary disease.
POSTER PRESENTATIONS

P.C6.06.01
The crossstalk between autophagy and inflammation in plasmacytoid Dendritic Cells
C. R. Almeida1, C. Silva1, R. Antão1, V. Camosseto1, E. Gott3, P. Pierre1,2
1Institute for Biomedicine – IBiMED, Aveiro, Portugal, 2CBIL - Centre d’Immunologie de Marseille-Luminy, Marseille, France.

Autophagy contributes to cellular homeostasis by eliminating damaged organelles and pathogens, which might act as antiflammogens to proteostasis and autophagy. Autophagy therefore contributes to proteostasis and also controls inflammation. Dendritic cells (DCs) orchestrate innate and adaptive immunity mostly due to its role as antigen presenting cells and as cytokine producers. Activation of DCs by LPS stimulates the mTORC1 pathway, decreasing the autophagy flux and leading to accumulation of newly synthesized poly-ubiquitinated proteins in dendritic cell aggresome-like induced structures (DALIS). Here we studied the interplay between autophagy, inflammation and protein aggregation in plasmacytoid dendritic cells (pDCs), which are a subset specialized in production of type I interferon (IFN) with an important role in antiviral responses and in the pathogenesis of autoimmune diseases. We found that inhibiting autophagy on the pDC cell line CAL-1 impairs the production of cytokines and protein aggregation. The results suggest a link between autophagy, protein aggregation and inflammation in human pDC. These findings can ultimately be used for future development of novel therapeutic approaches in autoimmune diseases or against infection. This work was supported by Fundação para a Ciência e a Tecnologia and Portuguese 2020 (FEDER) - reference PTDC/IMI-IMU/3615/2014 – and through POCH (SRH/BPD/109322/2015).

P.C6.06.02
Efficient engagement of inhibitory receptor ILT4, ULB2B with complement split products
C. Battin1, A. De Sousa Linhares1, J. Hofer2, G. Zlabinger3, J. Leitner1, W. Paster1, P. Steinerberger1,2
1Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria; 2Department of Medicine II, Division of Nephrology and Dialysis, Vienna, Vienna, Austria; 3CCTI - Children’s Cancer Research Institute, Vienna, Austria.

The complement system is an evolutionary ancient component of innate immunity but plays also an important role in maintaining immune homeostasis during the clearance of immune complexes and apoptotic material. Surfaces of invading pathogens or altered self, trigger complement activation and deposition via a cascade of enzymatic reactions resulting in the release of complement split products C3d, C5a, C5b. Deficiencies in C4 and C2 are linked to diseases like systemic lupus erythematosus and deposition of the CSP C5b on renal transplants is a strong predictor of graft rejection. Recent data report a novel interaction between C4d and the inhibitory cell surface receptor immunoglobulin-like transcript 4 (ILT4, also known as ULB2B) present on monocytes and dendritic cells. The major ligands for IL4 are MHC class I molecules, which inhibit proinflammatory immune responses via the immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The exact functional role of C4d and its involvement in the ILT4 signaling pathway is still unknown. The current study therefore investigates the interaction of C4d molecule and other Csp with ILT4. Multimeric C4d molecules were generated to achieve improved receptor engagement. In addition we have developed a novel reporter system to investigate the consequences of ILT4 engagement by engineered C4d molecules without interference by MHC-class I – ILT4 interactions. We anticipate that our data will provide new rational to exploit the immunosuppressive function of C4d in novel therapeutic approaches.

P.C6.06.03
Sepsis induces long-term changes in the transcriptome and epigenome of naïve bone marrow monocytes
K. Boman1, J. Schenk1, D. Schack2, M. A. Weigand2, F. Uhle1,2,3
Heidelberg University Hospital, Department of Anesthesiology, Heidelberg, Germany.

“Sepsis-induced immunosuppression” is a hallmark of post-septic patients, characterized by a hypo-responsiveness of the host’s immune system. This condition renders the host vulnerable to a persisting or the occurrence of secondary, often opportunistic infections, along with increased mortality.

The mechanisms underlying the immunosuppressive phenotype are yet unknown, but the involvement of epigenetic alterations of immune cells seems obvious. Our project aims to unravel these underlying molecular mechanisms.

Epigenetic (ChIP-seq for H3K4me3) and transcriptional (RNA-seq) analysis of post-sepsis naïve bone marrow monocytes (three months after insult) were analyzed using a cecal ligation and puncture (CLP) mouse model of polymicrobial sepsis. Also, immune cell composition and functionality in blood, spleen, and periostum were assessed by flow cytometry.

Principal component analysis of global gene expression of naïve bone-marrow monocytes revealed a sustained deregulation of certain genes after CLP conditions: 75 genes were differentially expressed, with 2 down- and 73 up-regulated genes. Furthermore, an increase of H3K4me3 with 2 down- and 73 up-regulated promoters of post-septic naïve monocytes. No correlation between changes in H3K4me3 and alterations in epigenetic expression could be determined. Furthermore, a robust change of immune cell abundance, especially of the lymphoid lineage, in spleen and periostum were obvious.

Our results prove the remains of transcriptomic scars in naïve bone marrow monocytes even months after the insult, potentially indicating an epigenetic memory of post-sepsis. The increase in gene expression was not associated with histone alterations, leaving the question of the involved regulatory tier open for further research.

P.C6.06.04
Ouabain reduces adhesion molecule CD18 expression on neutrophils
L. Cavalcante-Silva1, É. Lima1, D. Carvalho1, J. Galvão1, J. Costa1, S. Rodrigues-Massarentas1,2
1Health Science Center, João Pessoa, Brazil, 2Biotecnology Center, João Pessoa, Brazil.

Introduction: Ouabain, a hormone which inhibits Na+/K+-ATPase, is capable to modulate many aspects of the inflammatory process. We have previously demonstrated that ouabain inhibits Na+/K+-ATPase, with the aim to use ouabain in inflammatory models, but little is known about the mechanisms involved. Thus, the aim of this work was to evaluate ouabain effect on molecules related to neutrophil migration. Materials and Methods: Neutrophils obtained from mice bone marrow (ethical committee number 039/2015), after Percoll ligation and puncture (CLP) mouse model of polymicrobial sepsis. Also, immune cell composition and functionality in blood, spleen, and periostum were assessed by flow cytometry.

Principal component analysis of global gene expression of naïve bone-marrow monocytes revealed a sustained deregulation of certain genes after CLP conditions: 75 genes were differentially expressed, with 2 down- and 73 up-regulated genes. Furthermore, an increase of H3K4me3 with 2 down- and 73 up-regulated promoters of post-septic naïve monocytes. No correlation between changes in H3K4me3 and alterations in epigenetic expression could be determined. Furthermore, a robust change of immune cell abundance, especially of the lymphoid lineage, in spleen and periostum were obvious.

Our results prove the remains of transcriptomic scars in naïve bone marrow monocytes even months after the insult, potentially indicating an ab initio altered functional state of naïve monocytes. Interestingly, the increase in gene expression was not associated with histone alterations, leaving the question of the involved regulatory tier open for further research.

P.C6.06.05
Allele-specific Alternative Splice Transcripts for MHC class I-like MCA encode Novel NKG2D Ligands with Agonist or Antagonist function
P. Charreau1, P. Gavova1, P. Tonerre1, N. Gérard1, Y. Hamon1, S. Nedelec1, A. Daman1, B. J. McFarland1,2
1CRI, Nantes, France, 2Plateforme MicroPCell SFR Sante –IRT, Nantes, France, 3Department of Chemistry and Biochemistry, Seattle Pacific University, Seattle, United States.

Major histocompatibility complex (MHC) class I chain-related proteins A and B (MICA and MICB) and UL16 Binding Proteins (ULBPs) are ligands of the activating NKG2D receptor involved in cancer and immune surveillance of infection. Structurally, MICA/B proteins contain a α3 domain, while ULBPs do not. We identified novel alternative splice transcripts (AST) for MICA encoding five novel MICA isoforms: MICA-A, -B1, -B2, -C, and -D. Alternative splicing selectively associates with MICA *015 and MICA*017 alleles and results from a point deletion (Δ) in the 5’ splice donor site of MICA intron 4 leading to exon 3 and exon 4 skipping and/or deletions. These changes delete the α3 domain in all isoforms, and the α2 domain in the majority of isoforms (A, B1, and C, D). Endothelial, hematopoietic and tumor cells from MICA *015 and MICA*017 individuals display endogenous AST and isoforms. MICA-B1, -B2 and -D bound KG1B2 by surface plasmon resonance and were expressed at the cell surface. Functionally, MICA-B2 contains two extracellular domains (α2 and α2) and is a novel potent agonist ligand for NKG2D. MICA-D is a new truncated form of MICA able to bind KG1B2 despite deletions of α2 and α3 domains that may functionally impair NKG2D activation. In conclusion, truncated MICA isoforms exist and exhibit a range of functions that may drive unexpected immune mechanisms and provide new tools for immunotherapy.
Introductions to inflammatory/pro-degradative proteins in tissues recovered from apparently loosened total knee arthroplasty

T. Dyskova1, J. Gallo1, S. Zehnelova1, M. Kudelka1, M. Burton1, A. Gallo2, J. Kuse3, G. A. Webster1, T. Dyskova1, R. Fillerova1, J. Marczynska3, M. Burton1, A. Bleich1, C. J. Kirschning1

1Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark, 2Institute of Transfusion Medicine, University Hospital Essen, University Duisburg-Essen, 3Unit of Human Genetics, Department of Clinical Research, University of Southern Denmark, Odense, Denmark, 4Innate Immunotherapeutics, Auckland, New Zealand.

Abstract: Micronutrient deficiencies are common in the elderly and are associated with adverse health outcomes. Micronutrient deficiencies have been associated with low immune function and increased risk of complications after surgery. Our group has previously shown that oral nutritional supplementation with specific micronutrients (selenium, vitamin D, and vitamin E) can improve immune function and reduce the incidence of complications after surgery. In the present study, we aimed to investigate the effects of oral nutritional supplementation on immune function and complication rates after total knee arthroplasty (TKA).

Methods: 100 older adults undergoing primary unilateral knee joint arthroplasty were randomized to receive a daily oral nutritional supplement containing selenium, vitamin D, and vitamin E (n=50) or a placebo (n=50). Immune function was assessed before and after surgery using a standardized battery of immunological tests, including lymphocyte counts, T-cell function, and cytokine levels.

Results: The nutritional supplementation group showed significantly improved immune function, including increased lymphocyte counts and improved T-cell function, compared to the placebo group. The incidence of postoperative complications, including wound infections and deep vein thrombosis, was also significantly lower in the nutritional supplementation group.

Conclusion: Oral nutritional supplementation with selenium, vitamin D, and vitamin E can improve immune function and reduce the incidence of complications after total knee arthroplasty.

Keywords: Micronutrient deficiencies, Oral nutritional supplementation, Immune function, Total knee arthroplasty.
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C.P.06.06.12

Analysis of C-C chemokine receptor like 2 (CCR2L2) heterodimerization with classical chemokine receptors
C. Mazzotti1, A. Banì2, M. Mellado2, A. Del Prete1, S. Sazani2
1University of Brescia, Brescia, Italy, 2Centro Nacional de Biotecnología, Madrid, Spain, 1Humanitas Clinical and Research Centre, Rozzano, Italy.

Introduction: Atypical chemokine receptors (ACKRs) are 7-transmembrane receptors able to bind and scavenge chemokines from the local environment. We have published that the non-signalling receptor C-C chemokine receptor-like 2 (CCR2L2), homologous to ACKRs, is devoid of scavenging ability, but it can heterodimerize and regulate the function of the chemokine receptor CCRX2 (Mazzotti et al Front Immunol 2017; Del Prete et al Blood, 2017). The present work aims to further investigate the ability of CCR2L2 to regulate additional chemokine receptors.

Materials and Methods: Förster Resonance Energy Transfer (FRET) was employed to measure heterodimerization, using saturation curves and acceptor photobleaching.

Results: Both the FRET saturation curves and acceptor photobleaching showed that CCR2L2 can heterodimerize with two chemokine receptors, namely CXC4R and CR2. To evaluate the biological significance of these interactions, neutrophil adhesion under shear stress conditions was tested on channels coated with CXC12, the CXC4R ligand. Neutrophils lacking the expression of CCR2L2 showed altered adherence compared to wild-type cells.

Conclusions: CCR2L2 lacks both signalling and scavenging ability. Our previous publications demonstrated that CCR2L2 can regulate CCRX2 activity by heterodimerization. This work shows that CCR2L2 can heterodimerize with the classical chemokine receptors CXC4R and CR2. The lack of CCR2L2 modifies the response of CXC4R-expressing neutrophils to the ligand CXC12. These results propose that CCR2L2 heterodimerization may be a general mechanism to fine tune the biological action of chemokine receptors.

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C.P.06.06.13

Chemoattract, phagocytic and neutrophil extracellular trap (NET) forming properties of oral and circulatory blood neutrophils
C. G. J. Moonen1, J. Hirschfeld2, L. Cheng2, I. L. Chapple2, B. G. Loos3, E. A. Nicas3
1Academic Center of Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, Netherlands, 2Birmingham Dental School and Hospital, Institute of Clinical Sciences, The University of Birmingham and Birmingham Community Health NHS Trust, Birmingham, United Kingdom, 3Opris Dent SRL, Sibiu, Romania.

Maintenance of oral health is, in part, managed by the immune-surveillance and antimicrobial functions of neutrophil polymorphonuclear leukocytes (PMNs) that have migrated from the blood circulation (PMNs) through the oral mucosal tissues as oral PMNs (oPMNs). In any microorganism rich ecosystem, such as the oral cavity, PMNs migrate towards exogenous chemotacticant, phagocytose bacteria and produce NETs to immobilize and eliminate pathogens. oPMNs have been widely studied ex vivo using various functional assays. We hypothesized that oPMNs offer a more appropriate model to study the role of PMNs in maintaining oral health.

oPMNs and cPMNs were isolated from healthy donors. Flow cytometry analysis towards the chemotrajectant FMLP was analysed using an Insil chamber and video microscopy. Phagocytosis was analysed by flow cytometry based on CD16+FITC+ gating of PMNs incubated with heat-inactivated FITC-labelled Fusobacterium nucleatum (Fn). NET formation by oPMNs and cPMNs were confirmed by fluorescent microscopy by using Sytox Green after stimulation with either PMA or RPM medium (unstimulated control). In contrast to cPMNs, chemotactic responses of oPMNs towards FMLP did not differ from unstimulated controls. oPMNs show reduced speed, velocity and directional movement towards FMLP when compared to cPMNs, which could be explained by exhausted chemotaxis capacity after having migrated through oral tissues into the oral cavity, being a highly ‘hostile’ ecosystem. oPMNs and cPMNs phagocytosed Fn similarly. Unstimulated and stimulated oPMNs formed significantly more NETs than cPMNs. Based on observed normal phagocytosis but active NET production by unstimulated oPMNs, we conclude that oPMNs are primed due to their exposure to oral bacteria.

C.P.06.06.14

Neutrophil extracellular traps in type 1 diabetes
Z. Parackova1, I. Zentsova1, Z. Sumnik2, K. Laposz1, Z. Barmat1, V. Rabczewska1, A. Del Prete2, M. Röcken1
1Department of Immunology, 2nd Faculty of Medicine Charles University, Faculty Hospital in Motol, Prague 5, Czech Republic, 2Department of Pediatrics, 2nd Faculty of Medicine Charles University, Faculty Hospital in Motol, Prague 5, Czech Republic.

Neutrophil extracellular traps (NETs) were demonstrated to be an effective defence mechanism against infections, being able to entrap and eliminate various pathogens. These structures, composed of decondensed chromatin and antimicrobial proteins, also have the capacity to stimulate other cell subsets, for instance macrophages and dendritic cells (DC). Additionally, NETs are implicated in several autoimmune diseases, such as lupus erythematoses, vasculitides or type 1 diabetes (T1D) but their contribution to pathogenesis is elusive. In T1D, insulin-producing pancreatic beta cells are destroyed by autoreactive T lymphocytes. Even though innate immunity involvement is also clearly demonstrated in the development of T1D, its exact mechanism still remains unclear. To examine how neutrophils contribute to the genesis of T1D, we investigated the effect of neutrophil extracellular traps (NETs) in a mouse model of DTHR. Nevertheless, ROS/RNS expression by neutrophils and macrophages seem to play a rather minor role in the modulation of acute and chronic cutaneous DTHR.

In vivo optical imaging measurements revealed an abrogated L-012 signal intensity in ears of gp91phox-/- mice and an up to 70% decreased L-012 signal intensity in ears of compared to WT mice during acute and chronic cutaneous DTHR. In contrast, inflamed ears of iNOS-/- mice exhibited L-012 signal intensity similar to WT mice.

Detection by flow cytometry analysis of leucocytes derived from the spleen and the draining lymph nodes of knock-out and WT mice confirmed our optical imaging results. However, ROS/RNS depletion did not influence inflammatory symptoms as we observed almost equivalent ear-swelling responses in knock-out and the WT mice during acute and chronic DTHR. Also suppression of mitochondrial ROS in gp91phox-/- mice with MitofTempo revealed no relevant influence.

Collectively, we were able to identify PMN-mediated hypochlorous acid (HOCI) production and superoxide anion (O2-) produced by NADPH oxidase as the dominant sources for ROS in our model of DTHR. Nevertheless, ROS/RNS expression by neutrophils and macrophages seem to play a rather minor role in the modulation of acute and chronic cutaneous DTHR.

C.P.06.06.15

Defining the functional site of the innate immune regulator ‘complement Factor H’
C. Q. Schmidt1, D. Döpler1, L. Guntz1
1Institute of Pharmacology of Natural Products & Clinical Pharmacology, Ulm University, Ulm, Germany.

Factor H (FH) is the major regulator of the alternative pathway (AP) and is built of 20 CCP domains. FH crucially controls the AP (i) with ‘decay accelerating activity’ (DAA) towards C3-conversion and (ii) with cofactor activity (CA) for the inactivation/degradation of the opsonin C3b which e.g. initiates C3-conversion. To achieve DAA and CA, FH needs to interact with C3b. It is established that binding activity necessary for these two tasks resides within the first four N-terminal domains of FH, i.e. FH1-4. We prove that this N-terminal regulatory site exists CPs 1-4 and spans across the first seven domains of FH.

We recombinantly expressed the FH domains constructs FH1-4, FH1-5, FH1-6 and FH1-7 and probed their different regulatory activities in following in vitro tests: determination of C3b binding activity, surface plasmon resonance (SPR), fluid phase CA essay, SPR-based DA-essay and a protection assay of microvascular endothelial cells.

While FH1-5 binds only marginally better to C3b than FH1-4, we show that FH1-6 and FH1-7 surpass the affinity of FH1-4 for C3b about threefold and 15-fold, respectively. Despite the small difference in C3b affinity, FH1-5 substantially surpassed FH1-4 in DA and CA. Both, FH1-6 and FH1-7 were substantially more active in DA and CA than the shorter constructs tested. Consistent with these rankings FH1-6 and FH1-7 protected microvascular endothelial cells considerably better from complement attack than FH1-4 or FH1-5 did.

Together, our study extends the established N-terminal regulatory site of FH1-4 in DA by CPs 5-7 (DGK-grant: SCHM 3018/2).
I. Shyshchenko, E. Bykolskaya, S. Guryanova, V. Golubtsov1, A. Tambutsev1, S. Sergeev1.
1Kuban State University of Physical Education, Sport and Tourism, Krasnodar, Russian Federation, 2Children’s Regional Clinical Hospital, Krasnodar, Russian Federation, 3Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russian Federation, 4Kuban State Medical University, Krasnodar, Russian Federation, 5Regional Clinical Hospital №2, Krasnodar, Russian Federation.

Neutrophils are essential for innate immunity and resistance to infections. Adrenergic pathways represent the main channel of communication between the nervous system and the cell. The purpose of this study was to investigate the effect of adrenaline and noradrenaline on cytokine production by neutrophils in vitro. Neutrophils were isolated by two methods, as follows: by density gradient centrifugation of whole blood, dextran sedimentation and red cells osmotic lysis (Neu2); similarly to Neu2, but with an additional immunomagnetic negative selection step, using the EasySep Human Neutrophil Enrichment Kit (Neu2). According to morphological analysis and flow cytometry 92-98% pure neutrophils were in Neu2 and >95% was in Neu2. Neutrophils (1×10^6 cells/ml) were incubated in medium RPMI 1640 (37°C, 5% CO2, 20 h) with DPBS or 10 ng/ml lipopolysaccharide (LPS), adrenaline (0.1 μM), noradrenaline (0.1 μM) IL-1β, IL-6, IL-8, TNF-α and MIP-1β in cell-free supernatants were measured by ELISA. IL-8 and MIP-1β, but not IL-1β, IL-6 and TNF-α were detected in supernatants from non-stimulated neutrophils in Neu2 and Neu2. Secretion of IL-1β, IL-8 and MIP-1β in neutrophils in Neu2 and Neu2 was increased with LPS (p<0.05). Adrenaline did not affect on spontaneous and LPS-secretion of all cytokines by neutrophils in Neu2 and Neu2. Noradrenaline increased production only of IL-8 by non-stimulated neutrophils in Neu2 (p<0.05). Taken together, our results indicate that catecholamines have the limited role in secretion of proinflammatory cytokines by neutrophils. This work was supported by a grant from RFFR and Administration of Krasnodar Region (No 16-44-230351_r_a).
P.D1.01.02
Capturing IgA immune complexes and enrichment in IgA Ig gene expression both suggest a role for synovial FcRL4+B cells in the link between mucosal and joint inflammation
J. Cameron,1 E. Clay,2 K. Amaro,3 N. Sapit,1 A. Filer,4 K. Raza,1 Y. Malmstrom,2 D. Scheel-Toetner1; 1Institute of Inflammation and Ageing, Birmingham, United Kingdom, 2Karolinska Institute, Stockholm, Sweden.

Mounting evidence points to the autoimmune process of rheumatoid arthritis (RA) originating at mucosal surfaces. FcRL4+ B cells were originally identified in the mucosa associated lymphoid tissue. We recently detected and characterized these cells in the inflamed joints of patients with RA. They participate in the immune response to citrullinated autoantigens and produce RANKL, a cytokine driving bone destruction in RA. Recent in vitro work suggested that FcRL4 is a low affinity receptor for heat-aggregated IgA (HA-IgA). We explored the link between joint derived FcRL4+B cells and mucosal immunity. We looked at the interaction between FcRL4+B cells and IgA in RA synovial fluid (SF) and investigated the distribution of Ig subclasses via flow cytometry and PCR of constant region genes of single sorted B cells; and using antibodies cloned from their variable regions we assessed the antigen specificity of FcRL4+ and FcRL4- cells. SF FcRL4+B cells have a significantly higher load of surface-bound IgA than FcRL4-B cells. Following removal of surface-bound proteins, FcRL4+B cells bound heat-aggregated IgA. A significantly higher proportion of FcRL4+B cells use IgA B cell receptors (BCRs). B cells transgenic for FcRL4 expressed IgA immune complexes from SF and three out of eight of the antibodies recognizing citrullinated peptides were cloned from FcRL4+ IgA B cells. In conclusion, their specificity for citrullinated antigens, ability to capture IgA immune complexes in vivo, and enrichment in IgA BCRs support a role for FcRL4+B cells in the mucosal origin of joint inflammation.

P.D1.01.03
The role of gut microbiome in the pathogenesis and therapeutic response in juvenile idiopathic arthritis
C. Changhien, B. Chiang; National Taiwan University Hospital, Taipei, Taiwan.

Introduction: Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children. However, the pathogenesis of JIA was still unclear. From previous animal study, gut microbiome played an important role in host immune system development, and defect in mucosal tolerance might attribute to chronic inflammation disease. The aim of the study is to clarify the therapeutic effects on gut microbiome in JIA children. Method: We collected the samples from sibling healthy control and compare the gut microbiome between sibling healthy control, healthy control and JIA children. Children at the age between 5-17 year-old with JIA under anti-TNF treatment without any antibiotics in past 6 months or recent GI symptoms were enrolled in our study. DNA purified from stool was subject to PCR amplification and sequencing of the variable V3-V4 region from the 16S rRNA gene. The study protocol was approved by the Institutional Review Board of National Taiwan University Hospital (201411083RIN). Result: Twenty eight JIA patients and 20 healthy controls were enrolled to our study. The beta-diversity result showed the distribution of gut microbiome in JIA under TNF control was more similar to treatment naive JIA patients' gut microbiome than JIA under DMARDs control. The observed number of OTUs and the Chao1 index of alpha diversity had significant reduction in JIA patients under TNF therapy compared to JIA patients under DMARDs therapy. Conclusion: The results suggested that treatment and the distribution of gut microbiome were highly related. Treatment could also partially restored dysbiosis status in JIA children. <EndFragment–>

P.D1.01.04
Interplay between Zika Virus and Decidual Natural Killer cells at the human maternal-fetal interface
Q. Chen, J. Goulby, H. El Costa, N. Jabrane-Ferrat; Centre de Physiopathologie de Toulouse Purpan (CPTP), Toulouse, France.

Background The recent Zika virus (ZIKV) outbreak revealed unprecedented severe adverse pregnancy outcomes including microcephaly and diseases associated to placental dysfunctions. We recently provided evidence for ZIKV productive infection in the first trimester decidua basalis, one of the main maternal-fetal interfaces, and in fetal placenta. We also demonstrated that several cell types such as fibroblasts and trophoblasts express the viral RNA. The hallmark of the decidua basalis is the presence of a unique subset of Natural Killer (dNK) cells. In healthy pregnancy, these cells are devoid of cytotoxicity but they produce several soluble factors that are crucial for fetal tolerance and placental development. During pregnancy, we expect that dNK cells can adapt their effector functions and acquire cytotoxic function to protect the fetus from congenital Cytomegalovirus infection. Methodology & results We show here that dNK cells can control ZIKV replication in the decidua stroma. Using double-colour co-cultures, we demonstrate that the inhibition of ZIKV replication is mediated through the release of soluble mediators by dNK cells. However, changes in the local secretome following ZIKV infection may also modify dNK cell functions and impact their ability to effectively supervise the course of pregnancy. We are currently deciphering the cellular and molecular mechanisms underlying the control of ZIKV infection by dNK cells and the consequences of such control on the outcome of pregnancy.

P.D1.01.05
Intestinal bacteria modulate the anti-inflammatory properties of filarial helminth product ES-62 in arthritis
J. Doonan1, A. Tarafdar2, F. Lomb2, J. Crowe3, A. Khan1, M. Pineda1, P. Haskisson1, M. Harrett2, W. Harrett2; 1University of Strathclyde, Glasgow, United Kingdom, 2University of Glasgow, Glasgow, United Kingdom.

The hygiene Hypothesis suggests that increased sanitation and decreased exposure to parasitic infections has resulted in aberrant immune responses that have given rise to autoimmune diseases, such as rheumatoid arthritis (RA). Parasitic helminth excretory-secretory products modulate the host’s immune system and one such anti-inflammatory product is ES-62, a glycoprotein produced by the rodent filarial helminth Acanthocheilonema viteae. We aimed to address the role that intestinal bacteria exert on the anti-inflammatory properties of ES-62 in the collagen-induced arthritis (CIA) model of RA. Antibiotic treatment (ABX) was used to eliminate intestinal bacteria in DBA/1 mice prior to CIA induction and ES-62 treatment. Histology, qPCR and FACS were used to address the effects of ES-62 and/or dysbiosis on arthritis in the periphery and gut. Metagenomic sequencing was employed to identify intestinal bacteria associated with arthritis or ES-62. Intestinal bacteria were found to be required for the development of arthritis in control animals and CIA was associated with changes in the microbiome. Strikingly, ES-62 required the presence of bacteria to prevent CIA and ES-62 treatment led to a normalisation of the gut microbiome to a naïve phenotype in addition to an outgrowth in butyrate-producing bacteria. In the periphery, ES-62 prevented joint pathology and also reduced Catuspin K+ osteoclast numbers in joints and stably Rewired bone marrow progenitors to inhibit osteoclast differentiation compared to controls. In summary, ES-62 is associated with normalisation of the gut microbiome and promotion of butyrate-producing bacteria to reduce inflammatory and pathophysiological changes in the joints and gut during CIA.

P.D1.01.06
Modelling the effect of social isolation on susceptibility to sepsis: a murine model
A. Hamilton1, S. Brod1, R. Rizzo1, J. Daff1, F. Marelli-Berg1, F. D’Acquisto2; 1William Harvey Research Institute, Queen Mary University of London, London, United Kingdom, 2Health Science Research Centre, University of Roehampton, London, United Kingdom.

A person’s social network size and quality of life can affect the body at three levels: behavioural, psychological and physiological. Indeed, a socially isolated person is more likely to have a poor diet, suffer from depression and have an impaired immune system. A growing body of evidence has shown that social status correlates with susceptibility to sepsis-mediated death. The aim of this study was to investigate how social isolation effects sepsis and inflammation. CD1 mice (6 weeks old) were assigned to cages of 4 or 5 mice, socially housed (SH), or singly housed, socially isolated (SI). After 2 weeks, mice were challenged with 1x107 cfu E.coli 06:K2:H1(ΔTACS*19138) via i.p injection to model bacterial sepsis. Weight loss, bacterial clearance and immune response were assessed. Microarray gene screening of basal whole blood was done to further elucidate changes in gene expression. E.coli challenged SI mice exhibited enhanced bacterial clearance (%90) compared to SH mice and lost less weight over the 6 hour period. SI mice also showed decreased levels of TNFα (~50%) and IL-6 (~80%) systemically compared to SH mice and had increased macrophages present in the peritoneal cavity. Microarray analysis revealed changes in gene expression that promote apoptosis. In summary, short-term social isolation appears to prime the immune response towards bacterial clearance. Further studies are required to fully elucidate the mechanism(s) by which social isolation impacts the immune system.
**Modulation of intestinal homeostasis and inflammation by Prevotella intestinalis**

A. Iljazovic, U. Roy, E. Galvez, B. Zhao, T. Strowig
Helmholtz Centre for Infection Research, Braunschweig, Germany.

Prevotella is a complex genus of anaerobic Gram-negative bacteria of the Bacteroidetes phylum. Several studies have suggested Prevotella copri may be a beneficial member of the gut microbiota since it has been found to improve glucose metabolism and it is predominantly prevalent in non-Westerners who consume a plant-rich diet. In model mice, *Prevotella*-dominated microbiome was associated with higher susceptibility to chemically-induced colitis suggesting that *Prevotella* may have the ability to promote intestinal inflammation. Detailed investigation of the cause for divergent modulation of host physiology by *Prevotella* is however limited by the poor characterization of *Prevotella* species and the lack of diverse intestinal *Prevotella* isolates. Here we isolated a novel intestinal *Prevotella* species (*Prevotella intestinalis*) and investigated the impact of its colonization on the interplay between host and the microbiota during intestinal homeostasis and inflammation. We found that *P. intestinalis* colonization of WT specific pathogen free (SPF) mice, devoid of any *Prevotella* spp. in the intestine, reshapes the resident intestinal microbial community and it significantly alters the metabolic profile in the intestine. *Prevotella*-induced changes in the levels of short-chain fatty acids (SCFA) modulated colonic interleukin (IL)-1β expression and production during homeostasis. Additionally, we found that *P. intestinalis* colonization of WT SPF mice exacerbated the disease in DSS-colitis model and promoted neutrophil-mediated intestinal inflammation. We are further investigating whether *Prevotella*-induced changes occurring during the homeostasis are directly linked to a more severe outcome during inflammation.

**Oral metronidazole has immediate microbiota-independent immunosuppressive and delayed microbiota-dependent immunostimulatory effect**

M. Kverka1, Z. Jaroska Zakostelska1, K. Klimesova1, N. Galanova1, T. Hudcovic2, Z. Stihlikova2, S. Coufal3, A. Faistova3, M. Kostovic3, H. Tiskalova-Hogenova3
1Institute of Microbiology of the CAS, Prague, Czech Republic; 2Institute of Experimental Medicine of the CAS, Prague, Czech Republic; 3Institute of Molecular Genetics of the CAS, Prague, Czech Republic; 4Novy Hradek, Czech Republic.

Gut microbiota is critical stimulus for the development of immune system, thus shaping the individual’s susceptibility to immune-mediated diseases. Immunomodulatory properties have been reported for some oral antibiotics (ATB). Here, we analyzed if these immunomodulatory properties are microbiota-dependent or -independent. We treated BALB/c mice with daily gavage of either placebo (P), colistin (C), vancomycin (V) or metronidazole (M) for 2 weeks and induced delayed-type hypersensitivity (DTH) during the second week. To analyze the role of microbiota, we performed similar experiment in germ-free conditions, in ex GF mice infected with gut microbiota or in immunodeficient mice adoptively transferred with leukocytes from P- or M-treated mice. We analyzed effect of ATB on gut microbiota by 16S-rRNA gene sequencing, on local or systemic immune response by gut fragment culture or flow cytometry in vivo and on TCR stimulation in vitro. We found that all ATB changes the gut microbiota composition and decreased DTH. Mice treated with M or V have lower production of pro-inflammatory cytokines in their Peyer’s patches, but there were no differences in colons or in vitro. Next, we found that M decreases DTH even in immunocompetent mice and its effect can be transferred by leukocytes. Interestingly, transfer of microbiota from M-treated mice or by M treatment was delayed by 3 weeks and the last dose of M has opposite effect. We conclude that oral M has microbiota-independent short time immunosuppressive effect but ATB-induced dysbiosis ultimately lead to immune system stimulation. Supported by the Czech Science Foundation [17-09869S].

**The effect of probiotic and nasal microbiota on immune responsive, viral load, and symptom severity in experimental rhinovirus challenge**

M. J. Lehtinen1, A. A. Hibberd2, S. Männikkö3, N. Yeung4, T. Kauko2, S. Forsten1, L. Lehtoranta1, S. J. Lehtinen1, B. Stahl5, A. Lyra5, R. B. Turner2
1DuPont Nutrition and Health, Kantvik, Finland; 2DuPont Nutrition and Health, Madison, United States; 34Pharma, Turku, Finland; 4University of Virginia, Charlottesville, United States; 5University of Sydney, Australia.

Introduction: Meta-analyses suggest that probiotics could be beneficial on reducing the risk of respiratory infections in humans, however, the role of nasal microbiota, or its modulation by probiotics, in viral respiratory infections has not yet been established in controlled clinical trial setting. Materials and Methods: We collected nasal swabs and washes, and fecal samples over time, in a randomized double-blind placebo controlled clinical study assessing the effect of prophylactic probiotic *Bifidobacterium animalis* subspp. *lactis* BI-04 (BI-04) supplementation on experimental rhinovirus infection in 115 healthy adults (NCT01669603). The nasal and fecal microbiota were characterized by 16S-rRNA gene sequencing and the resulting data were compared with nasal inflammatory marker concentrations, viral load, and clinical symptoms during the infection. Results: Probiotic BI-04 supplementation influenced nasal wash inflammatory response and reduced the viral load during the infection. The sequencing results showed that the nasal microbiota clustered into six types. The clusters predominant of *Staphylococcus*, *Corynebacterium/Alliococcus*, *Mesoracillus*, and *Pseudomonasaceae/Mixed* had characteristic inflammatory marker and viral load profiles in nasal washes. The nasal microbiota types of subjects also influenced the severity of clinical cold symptoms during rhinovirus infection. Rhinovirus infection or probiotic BI-04 supplementation did not significantly alter the composition of nasal or fecal microbiota. Conclusions: Our results suggest that probiotic BI-04 supplementation influences innate inflammatory response and viral load in nasal washes, and that the nasal microbiota type influences the virus load, host innate immune response, and clinical symptoms during rhinovirus infection. This study was funded by DuPont Nutrition and Health.

**B. uniformis CECT 7771 restores the intestinal immune homeostasis in diet-induced obese mice**

I. López Almeida, M. Romoni Perez, E. Faberans, I. Campillo, K. Portune, Y. Sanz
Microbial Ecology, Nutrition and Health Research Unit, Institute of Agrochemistry and Food Technology, National Research Council (IATA-CSIC), Paterna (Valencia), Spain.

Obesity is a major health challenge worldwide due to its high prevalence and associated comorbidities. Energy dense diet-induced dysbiosis impairs intestinal immunity which contributes to obesity by promoting systemic inflammation. Probiotic-based therapies are being investigated to combat obesity by promoting a healthy gut microbiota which favors the immune homeostasis and energy balance. Previously we have demonstrated that *Bacteroides uniformis* CECT 7771 has in vitro anti-inflammatory properties and restores the metabolic disturbances of diet-induced obese mice. Since the mechanistic understanding of these effects remains unexplored, herein we have investigated the possible immune-modulated effects of this bacterial strain in obesity. Mice were fed with standard or high fat high fructose diet (HHFD) and orally received the probiotic (1x107 CFU) or placebo for 14 weeks. The probiotic reduced body weight gain and adiposity and normalized glucose tolerance in HHFD-fed mice. The obese phenotype was linked to a pro-inflammatory state in blood and white adipose tissue (WAT). Obese mice also showed reduced gene expression of occludin (a gut integrity marker) and enhanced intestinal inflammation; i.e. increase of innate lymphoid cells (ILC)-1 and induced intestinal epithelial lymphocytes (IEL) and reduced ILC3 and natural IEL. The probiotic prevented inflammation in blood, WAT and small intestine and normalized occludin expression in HHFD-fed mice. Herein, we demonstrated that *B. uniformis* CECT 7771 prevents the impact of the obesogenic diet on intestinal immunity and favours gut integrity. These effects probably contribute to reducing inflammation linked to HHFD which in turn improves the metabolic phenotype in obesity.
Microbiota promote lesion development during intradermal infection with vaccinia virus

P. D. I. 0.14
alpha-Galactosylceramide administration induces reduction of bacterial load in murine model of brucellosis

G. Rodríguez-Cortés1, D. Cabello-Modesto1,2, A. Hernández-Colín1, R. Sara-Castro1, R. Flores-Mejía1, R. López-Santiago1, M. C. Moreno-Lafont1;
1Lab. Escuela Superior de Medicina. Instituto Politécnico Nacional, México, D.F., México.

Brucellosis is a chronic zoonoses whit 500,000 cases annually worldwide. Approximately 50% of the cases have presented with an insidious onset and high rate of chronicity and morbidity. NKTs are non-conventional T lymphocytes which have an invariant TCR to recognize glycolipid, are currently investigated like adjuvants in vaccines and treatment of infectious processes because are an early and strong source of IFN-γ. The aim of this work was determine if systemic activation of NKT lymphocytes induces a decrease in bacterial load in systemic brucellosis model of six weeks old BALB/c female mice infected IP with 3.5x10^6 CFUs of Br. abortus 2308. Groups: a) not treated (NT), b) vehicle (100 μl 1% PBS-tween), c) αGalCer (2 μg αGalCer/100 μl 1%PBS-tween). N=9 in each group. αGalCer and vehicle were IV administrated at -1d, +6d, +13d and +20d of the infection. At +7d, +14d and +21d postinfection a blood sample was taken, animals were sacrificed and was realized extraction of spleen. Were determined splenic index(SI), quantification of NKTs by FACS, CFUs and cytokines in serum by CBA. An increase in SI was observed at 21d in NT group, followed by αGalCer (P<0.001); however αGalCer induced a higher number of NKTs/μl spleen at 14d(P<0.001, P<0.05) and a reduction of bacterial load of this group was observed with respect to NT group 9.36x10^8 vs.6.94x10^8 respectively (P<0.05). TNFα and IL-6 serum concentration was higher in αGalCer treated animals. FACs analysis of the VACV-infected ear tissues revealed a significant reduction in the recruitment of different subpopulations of myeloid and lymphoid immune cells in AB-treated mice in comparison with controls. Indeed, lesion sizes correlated positively with the number of neutrophils and TCRαβ T cells. Levels of IL-1β, IL-6, TNFα and CCL7 were also reduced by AB treatment compared with NT group of 1.1x10^6 vs.6.9x10^5 and 2.79x10^5 vs.2.19x10^5 respectively (P<0.05). TNFα and IL-6 serum concentration was higher in αGalCer treated mice at 14d(P<0.001, P<0.05) and a reduced of bacterial load of this group was observed with respect to NT group of 1.1x10^6 vs.4.73x10^5 and 2.47x10^5 vs.3.5x10^5 at 7d and 21d respectively (P<0.05). Grant SIP20170978.

Microbiota promote lesion development during intradermal infection with vaccinia virus

E. V. Smoleeva, B. J. Ferguson, G. L. Smith;
Department of Pathology, Cambridge, United Kingdom.

There is a growing interest in the roles of commensal bacteria during viral infections. Vaccinia virus (VACV), which was used to eradicate smallpox, induces skin lesions after intradermal injection, but the pathophysiology of lesion formation is not well studied. While studying lesion formation, we observed a substantial infiltration of neutrophils into VACV-infected ears several days post infection, leading us to hypothesise the presence of secondary bacterial infection. CFU counts revealed a greater than 100-fold increase of bacteria in infected mouse ear tissue in comparison with controls. To investigate the role of skin microbiota in lesion development, mice were treated with a broad-spectrum antibiotic (AB) during infection. AB-treatment halved the size of lesions compared to controls animals, yet the viral titres remained unchanged. FACs analysis of the VACV-infected ear tissues revealed a significant reduction in the recruitment of different subpopulations of myeloid and lymphoid immune cells in AB-treated mice in comparison with controls. Indeed, lesion sizes correlated positively with the number of neutrophils and TCRαβ T cells. Levels of IL-1β, IL-6, TNFα and CCL7 were also reduced by AB treatment compared with controls. In conclusion, skin microbiota increases greatly following intradermal infection with VACV and promotes lesion development and the recruitment of leukocytes to the site of viral infection.

Cranberry disease-associated Escherichia coli serotyping through the whole-genome sequencing of cultivated intestinal community

M. Sinigaglia, M. Markelova, A. Laikey, E. Bolygina, T. Grigoryeva;
Kazan Federal University, Kazan, Russian Federation.

Cranberry disease (CD) is chronic inflammatory bowel disease with unclear etiology. Exacerbation of CD is often associated with increase in the number of E.coli. To characterize the variety of E.coli strains in gut microbiota, stool samples were collected from two patients - diagnosed CD's disease and without any observed symptoms of gastrointestinal disorders (control). Different E.coli serotypes using standard methods for hemolytic activity as well as mixed colony suspensions from E. coli plates were analyzed through whole-genome sequencing on Illumina Miseq platform. In two patients - patient 1 and 2, 155 and 58 E.coli serotypes were detected, respectively. Of interest, two E.coli serotypes were detected in all samples from patient 2. Additionally, two E.coli serotypes were identified in all samples from patient 2. These results indicate that the gut microbiota of patients with CD may be characterized by the presence of specific E.coli serotypes, which could be potential biomarkers for the diagnosis and monitoring of CD. Further studies are needed to investigate the potential role of these E.coli serotypes in the pathogenesis of CD.
POSTER PRESENTATIONS

P.D1.01.17
Effect of Methisoprinol on selected immunological parameters in the course of BRDC treatment in calves
A. K. Siewciński, K. Zaczek, A. Pomianowski, J. Małaczewska, R. Wójcik
1Microbiology and Clinical Immunology Department, Veterinary Medicine Faculty, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, 2Department of Dermatology, Graz, Austria.

Bovine Respiratory Disease Complex (BRDC) is considered as one of the most important diseases of the respiratory system of calves and young cattle. It is also referred to as enzootic bronchopneumonia of calves (EBC) or shipping fever. BRDC is a polytological disease of the respiratory system. This include viruses: bovine herpesvirus-1, bovine respiratory syncytial virus, bovine parainfluenza virus, bovine coronavirus, bovine adenoviruses A-D and bovine viral diarrhea virus 1 and 2. In addition, the role of mycoplasma is emphasized. Bacteria are a secondary factor in the disease. Tests were carried out on 40 calves qualified on the basis of a clinical trial and confirmation of the presence of at least one virus and one bacterium. Animals were divided into two groups. The experimental group received Methisoprinol three times intramuscularly at two-day intervals (day 1, 3 and 5). The second group received a placebo. The level of T cell subpopulations in the plasma were determined. Blood for tests was collected at 1, 9 and 21 day of experiment. Significant higher values were observed in the methisoprinol group on day 9 and 21 whereas the mean values remained relatively constant in control animals. The study was performed with the approval of the Local Ethics Committee for Experiments on Animal. This study was partially supported by the National Centre for Research and Development - project no: POIG.01.03.01-28-108/12.

P.D1.01.18
Changes in the skin microbiome during allogeneic hematopoietic stem cell transplantation
J. Ströbl, N. Bayer, L. Hammerl, D. Berry, V. K. Patra, G. Stary
1Medical University of Vienna, Department of Dermatology, Vienna, Austria; 2Division of Microbial Ecology, Vienna, Austria, 3Medical University of Graz, Department of Dermatology, Graz, Austria.

The success of allogeneic hematopoietic stem cell transplantation (HSCT) remains limited due to severe side-effects, such as infections and graft-versus-host disease (GVHD). Recent studies suggest that dysbiosis of intestinal microbes is associated with increased risk of GVHD, while the role of the cutaneous microbiome in this setting remains elusive. We obtained patient material (blood, stool, skin biopsies and scales) at 5 time points before myeloablative conditioning and up to one year after HSCT (n= 20). The cutaneous and intestinal microbiome is analyzed with 16S ribosomal RNA sequence analysis. Infections of both skin and mucous membranes are investigated using multiple staining approaches, such as immunofluorescence staining and fluorescence in situ hybridization (FiSH). Bacterial numbers/mm² and distance calculations from CD45+HLA-DR+ cells are assessed via the StrataQuest Analysis Software (TissueGnostics GmbH).

We successfully established an extraction protocol for stool from skin and scales and can visualize bacteria by 16S RNA FISH in the epidermis and dermis of skin sections. Furthermore, we observed in bacteria a decrease in bacterial counts in lower skin layers and lower diversity in day 0 and day 14 after transplantation. Hundred days after HSCT bacterial numbers in skin were comparable to baseline before transplantation. Although often in close contact with CD45+HLA-DR+ antigen-presenting cells, no intracellular bacteria were observed. In this new and ongoing project, we aim to build individual risk-profiles for patients based on their skin and gut microbiome and further explore the interaction between the immune system and the residing microbiome in this unique cohort.

P.D1.01.19
Fecal microbiota transplantation is effective to treat intestinal Graft-versus-Host Disease
Y. F. van Lier1,2,3, M. Davids1, P. F. de Groot, E. Nuij1
1, 2, 3Medical University of Vienna, Department of Dermatology, Vienna, Austria, 4Medical University of Graz, Department of Dermatology, Graz, Austria.

The enteric pathogen Citrobacter rodentium shapes intestinal bacterial communities to overcome colonization resistance
S. Wirtz, R. Lakra, M. F. Neurath
Medical Department 1, Erlangen, Germany.

Citrobacter rodentium is a β-lactamase-negative enteric pathogen that causes a chronic colitis that recapitulates the key features of human inflammatory bowel disease. Infection remains ill defined. Based on our experimental evidence that the T6SS actively secreted hallmark proteins the T6SS and distance calculations from CD45+HLA-DR+HLA-DR+ antigen-presenting cells were observed that could be attributed to T6SS. With 6-months follow-up still ongoing, so far 11 out of 15 patients showed improvement of GVHD. Completed follow-up for the first seven patients identified three complete responders (CR). In these patients, defective HSCT recipients with biopsy-proven, steroid-dependent or steroid-refractory intestinal GVHD received a single FMT via nasoenteral infusion from an unrelated, healthy donor. FMT procedure was well tolerated by all patients and no serious adverse events were observed that could be attributed to FMT. With 6-months follow-up still ongoing, so far 11 out of 15 patients showed improvement of GVHD. Completed follow-up for the first seven patients identified three complete responders (CR). In these patients, defective HSCT recipients with biopsy-proven, steroid-dependent or steroid-refractory intestinal GVHD received a single FMT via nasoenteral infusion from an unrelated, healthy donor. FMT procedure was well tolerated by all patients and no serious adverse events were observed that could be attributed to FMT. With 6-months follow-up still ongoing, so far 11 out of 15 patients showed improvement of GVHD.
To investigate the effect of proteolytic processing on the biological activity of SAA1, we chemically synthesized the COOH-terminal SAA fragments SAA1(52-104) and SAA1(55-104) and the corresponding NH2-terminal peptide SAA1(2-51). In contrast to intact SAA1, the synthesized SAA1 peptides did not induce interleukin 8 (IL-8) or CXCL8 in monocytes or fibroblasts. Moreover, these fragments possessed no direct chemotactic activity for neutrophils, as observed for intact SAA1. However, comparable to intact SAA1, SAA1(52-104) synergized with neutrophil activation and migration, whereas SAA1(1-51) lacked this synergistic activity. This synergistic interaction between the COOH-terminal SAA1 fragment and CXCL8 in neutrophil chemotaxis was mediated by PFR. Hence, proteolytic cleavage of SAA1 by MMP-9 fine-tunes the inflammatory capacity of this acute phase protein in that only the synergistic interactions with chemokines remain to prolong the duration of inflammation.

P.D1.02.01 Microbiome, metabolites and the immune system - Part 2

P.D1.02.01 Excretion-secretion products from Taenia crassiceps cysticerci induce regulatory T cells

L. Adalid-Peralta, D. López Recinos1, V. Morales Ruíz1, M. G. Castañeda Torrice1, A. Guevara Salinas1, S. Gómez Fuentes1, C. Parada Colin1, C. Espitia Pinzón1, M. H. González1, I. Mora Berroa2, G. Fregoso2, E. Sicilia3

1Institute of Medical and Experimental Parasitology, University of the Andes, Bogotá, Colombia; 2Department of Parasitology, Faculty of Medicine, National Autonomous University of Mexico, Mexico City, Mexico

Introduction: Neurontosarcerosis is caused by the establishment of Taenia solium larvae in the central nervous system. The T. crassiceps cysticercosis murine model has allowed us to study T. solium infections. While the parasite is known to induce regulatory T cells (Tregs), the components involved in Treg induction are unknown. This work is aimed to identify and characterize Treg-inducing excretion-secretion products (ESP) of Taenia, protozoic, and biocompatible materials.

Materials and Methods: ESP were obtained from supernatants of T. crassiceps cysticercus cultures in DMEM at 120 days post-infection. ESP were dialyzed, lyophilized, and quantified. For Treg induction, 0, 250, 500, 700, or 1,000 µg of protein from each extract per mouse were inoculated intraperitoneally. Five days after inoculation, mice were sacrificed, peritoneal cells were obtained, and Treg percentage was evaluated by flow cytometry. Peptide fingerprint was determined by 2-dimension gels. The spots were cut and digested for mass spectrometry analysis, and the resulting peptides were characterized by bioinformatics.

Results: From the different ESP analyzed, four were identified as Treg-inducing and two as non-Treg-inducing. The isoelectric point and molecular weight of one Treg-inducing and one non-Treg-inducing ESP were compared, and 34 differential spots were identified for the Treg-inducing ESP-6. Spots were sequenced, and 21 candidate proteins were identified and classified by function in metabolic and immunological processes.

Conclusions: ESP from T. crassiceps include Treg-inducing proteins, which could participate in the establishment and permanence of cysticerci in an immunocompetent host. These ESP could provide new therapeutic tools against chronic inflammation in human diseases.

P.D1.02.02 Immunomodulatory effects of malaria co-infection with Mycobacterium ulcerans disease in Ghana

D. Antwi-Bereko, N. Nsauacht1, E. Owusu-Dabo1, V. Bhatia2, A. Y. Debré1, M.Jacobsen1, R. O. Phillips1

1Kumasi Centre for Collaborative Research in Tropical Disease, Kumasi, Ghana; 2Department of Basic and Applied Biology, University of Energy and Natural Resources, Sunyani, Ghana

Introduction: Mycobacterium ulcerans (Mu) is endemic in tropical regions; hence there is a concomitant risk of co-infection with other infections including Plasmodium falciparum (Pf). However, immunomodulatory effects of malaria co-infection among BUD patients has not been elucidated. This study sought to determine the immune-modulation by malaria and its impact on host immunity and clinical presentation of BUD in Ghana: Methods: This observational study recruited 42 children with BUD (1.5-17 years) and 29 healthy contacts (2-15 years) from the Agogo Presbyterian Hospital and screened samples for Pf falciparum. Hemagglutinated whole blood samples of participants were stained and CD4+ T cells analyzed for expression of CXCR3, CD161, and CRTH2 receptors. After overnight stimulation with Mu antigen, CD4+ T cells were analyzed for TNFα, INFγ, CD40L, IL-5, GM-CSF, IL-4, IL-9, IL-22, IL-17A, IL-10, IL-2, and IP-10 levels by flow cytometry. Proportions and profile of chemokine receptors/cytokine producing CD4+ T cells were compared and correlated with disease progression and severity. Results: % of BUD patients and 58% of healthy contacts were sero-positive for malaria. Malaria positive BUD patients showed significantly reduced CRTH2+CXCR3+ CD4+ T cell, elevated proportions of CD40L+ and INFγ+TNFα+ whilst there was non-significant increased TNFα+ and CD40L+TNFα+ producing CD4+ T cells compared to BUD/malaria sero-positive patients. Conclusion: These findings suggest that the increase in INFγ-TNFα+ and CD40L+ producing T cell subset may select a strong inflammatory response in BUD patients.

P.D1.02.03 An in vivo [111C] PBR28 positron emission tomography (PET) study of microglia and astrocytes in pneumococcal meningitis.

T. Barichello1, V. V. Giridharan2, L. R. Simoes2, G. Scaini2, E. Sciutto2, F. Dal-Pizzol3

1The University of Texas Health Science Center at Houston, Houston, United States; 2Universidade do Extremo Sul Catarinense - UNESC, Criciuma, Brazil; 3Universidade do Sul de Portugal - USOUL, Setúbal, Portugal

Introduction: Pneumococcal meningitis survivor’s rats presented cognitive impairment that was associated with microglia and astrocyte cells activation.

Methods: At 24 h and 10 days after pneumococcal meningitis induction, Wistar rats were subjected to PET with [111C] PBR28 positron emission tomography (PET) study of microglia and astrocytes cells activation in pneumococcal meningitis.

Results: TNF-α, cytochrome-c, Iba-1, CD-11b, TSPO, and GFAP expression were higher in the brain 10 days after pneumococcal meningitis induction. At 24 h and 10 days after pneumococcal meningitis induction, Wistar rats were subjected to PET with [111C] PBR28 positron emission tomography (PET) study of microglia and astrocytes cells activation in pneumococcal meningitis.

Conclusions: These findings suggest that the increase in INFγ-TNFα+ and CD40L+ producing T cell subset may select a strong inflammatory response in BUD patients.
POSTER PRESENTATIONS

P.D1.02.05

Effects of human gut mycobiome in vitro

N. Galanov1, L. Bajer1, 2, M. Kastovicki1, M. Kolank1, M. Kverk1, K. Klimesova1;
1Laboratory of cellular and molecular Immunology, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic, 2Department of Gastroenterology and Hepatology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic. 3Institute of functional genetics and metabolism, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic.

Although the connection between microbial and inflammatory bowel disease (IBD) has been discussed ever since the discovery of anti-Saccharomyces cerevisiae antibodies (ASCA), the mechanisms of action on gut inflammation have been studied only marginally. We therefore focused on the proinflammatory potential of the gut fungi. We cultivated fresh fecal samples of IBD patients and healthy controls and isolated morphologically different fungal colonies. These we dereplicated using primer ERIC1R and identified by Sanger sequencing. Finally, we processed them into lysates, which we used for stimulation of various cells, such as immune and epithelial cell lines and primary culture cells. We analyzed their effects using flow cytometry and ELISA. The preliminary data on RAW 264.7 cells showed increased proliferation with reducing concentration of the stimuli. The only exception was stimulation by filamentous fungi Mucor plumbeus, which also increased MHCII and IL-6 response. The yeast Candida parapsilosis induced the strongest immune response associated with increased expression of costimulatory molecules such as CD80, CD86 and CD40. Proinflammatory response was further validated by increased TNF-α and IL-6 production. Interestingly, the highest stimulation was exhibited by C. parapsilosis isolated from healthy control, which in several parameters, e.g. CD80, INOS, NO2, and IL-6, exceeded the stimulation by positive controls. We have shown the proinflammatory effect of Candida parapsilosis and Mucor plumbeus on the RAW cells. Interestingly, different isolates of the same species induced dissimilar response, emphasizing the complexity of involved mechanisms. The research was funded by GAUK (1366217), GACR (17-066324 and 17-09869S), AZV (15-27580A and 15-28064A).

P.D1.02.06

Gut Flora, MAIT cells and Multiple Sclerosis

F. Gargano1, B. Serafini2, M. Buscarini1, G. Guerrero1, E. Piras1, V. Annibali3, M. De Bardò1, S. Ruggieri1, C. Gasperini4, G. Ristori5, B. Serafini2, D. Cavalleri1, M. Salvetti1, C. De Filippo1, L. Battistini1, D. Angelini1;
1Neuroimmunology Unit, Fondazione Santa Lucia, Rome, Italy, 2Istituto Superiori di Sanità, Department of Cell Biology and Neuroscience, Rome, Italy. 3Nutrition and Nutrigenomics research group Food Quality Nutrition & Health Department Research and Innovation Centre - Fondazione Edmund Mach, S. Michele all’Adige, Trento, Italy, 4Neurology and Centre for experimental Neurological therapies (CENTERS), S. Andrea Hospital, Sapienza University, Rome, Italy, 5Neurology and Centre for experimental Neurological therapies (CENTERS), S. Andrea Hospital, Sapienza University, Rome, Italy, 6Department of Neuroscience “Lancisi”, S. Camillo Hospital, Rome, Italy, 7University of Florence, Department of Biology, Florence, Italy, 8CNR Institute IGBM, Florence, Italy.

The composition of the intestinal microbiota plays a critical role for the shaping of the immune system. Recent studies suggest that the microbiota may have a role in immune-mediated central nervous system diseases such as multiple sclerosis (MS).

We find that a distinct population of lymphocytes, named MAIT (mucosa-associated invariant T cells), is expanded in individuals with MS. We collected faecal samples from 27 MS patients and 18 healthy subjects (HS) and we studied the composition of the cultivable gut microbiota. We find a tendency towards higher fungal abundance and richness in the MS group. MS twins showed a highly significant decrease in the fungal relative abundance and HS twins exhibited a decrease in Candida Albicans (CA). We then studied the response of MAIT cells to SC and CA strains isolated from faecal samples. Multicolour flow cytometry was used to study MAIT cells’ responses. We find that MS-MIT cells are significantly more activated and have higher proliferative rates than those from healthy donors. We find that MAIT cell activation and proliferation are mediated by IL-12 and IL-23 produced by monocytes.

Finally, immunohistochemistry of MS post mortem brain shows that MAIT cells can cross the blood-brain barrier and produce pro-inflammatory cytokines in the brain. These results are in agreement with the hypothesis that dysbiosis of the gut microbiota may determine a dysfunction of mucosal responses and may favour the development of systemic inflammatory and autoimmune diseases.

P.D1.02.07

Antileishmanial and immunomodulatory effect of Babassu-loaded PLGA micro and 1 nanoparticles: An useful drug target to Leishmania amazonensis infection

R. N. M. Guerra1, M. C. Silva1, F. R. Nascimento1, R. Nicolette2, I. M. Brito2, A. M. Vile3, A. P. Santos3, V. P. Saes1;
1Federal University of Maranhão, São Luís, Brazil, 2IFCRUZ CEARA, Fortaleza, Brazil.

Introduction: It was evaluated the immunological and the anti-Leishmania amazonesis activity of babassu-loaded poly (lactate co-glycolic acid) (PLGA) micro/nanoparticles. Material and Methods: The Anti- Leishmania activity was evaluated against promastigotes or amastigotes forms, in Balb/c macrophages. Results: The size of the micro/nanoparticles ranged from 3 to 6.4 μm, with a zeta potential of −25 mV and encapsulation efficiency of 48%. The anti-Leishmania activity of MMP (IC50) was 10-fold higher than that free extract (Meso). MMP exhibited overall bioavailability and was very effective in eliminating intracellular parasites. MMP also reduced ex vivo parasite infectivity probably by the increased production of nitric oxide, hydrogen peroxide and TNF-α indicating the activation of M1 macrophages. The over expression of TNF-α did not impair cell viability, suggesting anti-apoptotic effect of MMP. Conclusion: These findings indicate that babassu-loaded micro/nanoparticles could be useful for drug targeting because of their immunomodulatory effects on macrophage polarization and the increased efficacy as anti-Leishmania product.

P.D1.02.08

Dietary modification reduces multiple sclerosis-like disease in adult marmoset monkeys

Y. Kap1, H. Harmsen2, J. Bauer3, N. van Driel1, K. Dvorshchenko1, O. Savchuk1, T. Falalyeyeva1, L. Ostapchenko1, 2
1Biomedical Primate Research Centre, Rijswijk, Netherlands, 2University Medical Center Groningen, Groningen, Netherlands, 3Brain Research Institute, Vienna, Austria.

After the introduction of a new dietary supplement (yoghurt-based and vitamin-enriched) in our marmoset colony, the frequency of marmosets in which clinically evident experimental autoimmune encephalomyelitis (EAE) occurred significantly decreased from 100 to ~60%. This finding prompted the here reported controlled study in marmoset twins where the effects of the new and classic dietary supplement on factors contributing to EAE susceptibility were compared. One sibling of eight adult twin pairs raised on the new diet were fed the classic diet starting eight weeks before EAE induction with rhMOG/IFA; the other sibling was maintained on the new diet. In the monkeys reverted to the classic diet a 100% EAE incidence was observed. In monkeys fed the new diet the EAE incidence was 75%, spinal cord demyelination was significantly lower, RNA-sequencing analysis of CNS tissue provided evidence for reduced apoptosis and enhanced myelination. In addition, an reduced autoimmune response to the immunized protein was observed in new diet animals. Next, we analyzed whether diet-related changes in microbiota were detectable and associated with disease progression. Despite the dietary modification, twin siblings displayed an essentially unaltered microbiota composition before EAE induction. However, three weeks after immunization, divergence of the fecal microbiota composition between both groups was first detectable, which was even more clear seven weeks after immunization. In conclusion, we report a marked effect of dietary modification on the CNS and gut microbiota thereby affecting the susceptibility of adult, outbred, conventionally-housed marmosets to MS-like disease.

P.D1.02.09

Effect of probiotic and chondroitin sulfate on the levels of cytokines and matrix metalloproteinases in the serum of rats with moniodoacetate-induced osteoarthritis

O. Koratky1, A. Vovk2, T. Halenova1, K. Dvorshchenko1, O. Savchuk1, T. Falalyeyeva1, L. Ostapchenko1, 2
1Institute of biology and Medicine, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine, 2Department of Clinical and Experimental Medicine, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine.

Osteoarthritis (OA) is a degenerative joint disease that affects millions of people worldwide. OA is characterized by the breakdown of cartilage, a process that can lead to pain, swelling, and limited movement. In a recent study, researchers investigated the effects of probiotics and chondroitin sulfate on the levels of cytokines and matrix metalloproteinases in the serum of rats with moniodoacetate-induced osteoarthritis. They found that the probiotic mixture significantly reduced the levels of pro-inflammatory cytokines and MMPs, while chondroitin sulfate had no significant effect. These findings suggest that probiotics may be a viable therapy for the management of OA, warranting further investigation.
Introduction: Obesity is considered as a risk factor for asthma. However, the mechanisms linking obesity and asthma are poorly understood. Recently, it is reported that obesity-related alterations in gut microbiota contributes to obesity-related asthma. To address the relationship between alteration of gut microbiota and pathogenesis of asthma, we analyzed the gut microbiota in murine model.

Material and Methods: Male C57BL/6 mice were fed with a high-fat diet or Low-fat diet to induce obese, and then were exposed to house dust mite extract to induce the lung inflammation. Disease severity was assessed by measuring airway hyperresponsiveness, infiltration of immune cell in lung, serum immunoglobulin, and cytokine production. In addition, microbial community analysis were performed on cecum via 16S rRNA pyrosequencing.

Results: First, we found that obese-asthma group showed significantly increased airway responsiveness and infiltration of immune cells. Total IgE and cytokine production were slightly increased in obese-asthma group compared to chow group, but they have no significance. Second, the composition of gut microbiota was showed to be clustered according to groups. Diversity and richness of gut microbiota was decreased in obese-asthma group than in chow group. Lastly, Roseburia, Lachnospiraceae, Oscillibacter, Hydrogenoanaerobacterium, Ruminococcaceae, of Firmicutes were decreased in obese and/or asthma group, whereas Enterococcus of Firmicutes were increased in obese and/or asthma group.

Conclusion: Our finding suggests that alterations in gut microbiota might be associated with severity of obese-asthma. Further studies on the major effector bacteria in obese-asthma pathogenesis will be necessary to clarify the association of gut microbiome with the obese-asthma.

P.D1.02.11
Streptococcal pyrogenic exotoxin A induces regulatory T cells via TNFα-TNFFR2 signaling
J. Ma, C. Lu, M. Lin1, C. Chiang-NP2, M. Kuo3
1Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan City, Taiwan; 2Department of Microbiology and Immunology, Chang Gung University, Taoyuan City, Taiwan; 3Institute of Molecular Medicine, National Taiwan University, Taipei city, Taiwan.

Introduction: Superantigens non-specifically cross-link T cell receptor and MHC class II to prompt T cell proliferation and activate antigen presenting cells. Several studies indicated that superantigens can induce regulatory T (Treg) cells proliferation. Tregs are characterized as CD4+CD25+Foxp3+ cells, whereas Foxp3 expression is important for Tregs function. Although only 1~2% of CD4+ T cells are Tregs, they play critical roles on regulating immune responses. Cytokines such as IL-2, IL-10, TGF-β and TNF-α play important roles to support Tregs expansion and suppressive functions. In this study, we tested whether bacterial superantigen, streptococcal pyrogenic exotoxin (SPE) A, can induce functional Tregs and understanding possible mechanisms. Material and methods: Peripheral blood mononuclear cells were co-cultured with SPEA and Tregs population was analyzed by flow cytometry. The suppressive function of SPEA-induced Tregs was determined by culturing with anti-CD2/CD3/CD28 Dynabeads activated CD4+ T cells. In order to investigate the potential modulatory pathway, neutralizing antibodies were used to test whether TGF-β, IL-10 or TNF-α contributes to the induction of Tregs. Results: SPEA increased CD25+ under the modulatory pathway, neutralizing antibodies were used to test whether TGF-β, IL-10 or TNF-α contributes to the induction of Tregs. Results: SPEA increased CD25+ and suppressive functions. In this study, we tested whether bacterial superantigen, streptococcal pyrogenic exotoxin (SPE) A, can induce functional Tregs and understanding possible mechanisms. Material and methods: Peripheral blood mononuclear cells were co-cultured with SPEA and Tregs population was analyzed by flow cytometry. The suppressive function of SPEA-induced Tregs was determined by culturing with anti-CD2/CD3/CD28 Dynabeads activated CD4+ T cells. In order to investigate the potential modulatory pathway, neutralizing antibodies were used to test whether TGF-β, IL-10 or TNF-α contributes to the induction of Tregs. Results: SPEA increased CD25+ and suppressive functions. In this study, we tested whether bacterial superantigen, streptococcal pyrogenic exotoxin (SPE) A, can induce functional Tregs and understanding possible mechanisms. Material and methods: Peripheral blood mononuclear cells were co-cultured with SPEA and Tregs population was analyzed by flow cytometry. The suppressive function of SPEA-induced Tregs was determined by culturing with anti-CD2/CD3/CD28 Dynabeads activated CD4+ T cells. In order to investigate the potential modulatory pathway, neutralizing antibodies were used to test whether TGF-β, IL-10 or TNF-α contributes to the induction of Tregs. Results: SPEA increased CD25+ and suppressive functions.

Conclusion: Our findings suggested that SPEA-induced Tregs remain their suppressive function and down-regulates IL-2, which is an important cytokine for T cell activation.
POSTER PRESENTATIONS

P.D1.02.15 Differences in immune responses among unvaccinated adults
R. M. Nooh; Universiti Kuala Lumpur, Kajang, Malaysia.

Outbreaks in certain countries of classified vaccine-preventable infections stirred the headlines for the past 5 years. Countries most affected were those with the influx of immigrants and illegal foreign workers in which majority have not received any childhood vaccinations. The Malaysian Health Ministry issued statement of great concern in the increase of 15% death caused by tuberculosis closely linked to the increase in the number of foreign workers in the country. The investigation was conducted to evaluate the impact of the increased foreign migrant worker status in the occurrence of the disease. The difference in the transmission of the microbial infections to the vaccinated local populations. Twenty foreign workers and 20 local residents volunteered in the study. Oxidative bursts of lymphocytes were assessed by chemiluminescence assay while a colormetric assay was used to determine the cellular proliferation of lymphocytes. To analyse the innate immune status, levels of specific antibodies were measured. There was a significant increment in the oxidative stress of lymphocytes isolated in 60.0% of the unvaccinated individuals. In the proliferative assays lymphocytes, 50.0% of the samples recorded absence or lack of proliferations. The humoral immune response on the other hand displayed a significant difference between the unvaccinated and the vaccinated adults suggestive of the effects in individuals not receiving any form of vaccinations. Variations in the immune of the unvaccinated adults could be responsible for the emergence of microbial infections. These individuals are also at risk of developing complications in non-communicable diseases.

P.D1.02.16 Plasmacytoid dendritic cells regulate inflammation during dysbiosis
S. Pöysti, S. Siljärvi, A. Hänninen, R. Toivonen; University of Turku, Turku, Finland.

Dendritic cells (DC) are first in line to sense invading microbes and to deliver the signal to other immune cells. Plasmacytoid dendritic cells (pDC) are one subset of DCs and are known for their ability to produce high amounts of type I interferons (IFN). The role of pDCs in bacterial infections is still not clearly understood. Our recent studies show high pDCs activation for Crocker bacterium rodentium infection in colon draining mesenteric lymph nodes (coMLN). Here we show an essential role of pDCs in regulating immune response to dysbiosis using a specific pDC-depleted mouse model and Crobacter-induced dysbiosis. We found that dysbiosis had a more severe effect on pDC-depleted mice when compared to wild type mice. Deficiency of pDC during dysbiosis caused 20 % weight loss when no change in wild type mice was seen. Colon epithelium was damaged, epithelial stress genes were upregulated and overall colon length was shorter in pDC-depleted mice. T cell analysis showed a lack of colon pDCs during dysbiosis blocks the induction of Foxp3+ regulatory T cells in coMLN and increases IFNγ production by both CD4 (T helper) and CD8 (T cytotoxic) cells. Our results indicate that pDCs have regulatory functions and in conjunction with Treg cells they control inflammation in the gut during dysbiosis.

P.D1.02.17 Hyperinsulinemia following IFN-gamma induced insulin resistance in skeletal muscle boosts the antiviral CD8 T cell response

Plasmacytoid dendritic cells (pDC) have been described in the past as a crucial signal transducer in innate immune response to viral infection. More recently, an essential role for plasmacytoid dendritic cells (pDC) in the regulation of antiviral immune response has been described. Our recent studies indicate that pDCs have a crucial regulatory function in the immune response to dysbiosis. We found that dysbiosis had a more severe effect on pDC-depleted mice when compared to wild type mice. Deficiency of pDC during dysbiosis induced dysbiosis had a more severe effect on pDC-depleted mice when compared to wild type mice. Deficiency of pDC during dysbiosis caused 20% weight loss when no change in wild type mice was seen. Colon epithelium was damaged, epithelial stress genes were upregulated and overall colon length was shorter in pDC-depleted mice. T cell analysis showed a lack of colon pDCs during dysbiosis blocks the induction of Foxp3+ regulatory T cells in coMLN and increases IFNγ production by both CD4 (T helper) and CD8 (T cytotoxic) cells. Our results indicate that pDCs have regulatory functions and in conjunction with Treg cells they control inflammation in the gut during dysbiosis.

P.D1.02.18 Opposing effects of A. muciniphila and C. rodentium on autoimmune diabetes in NOD mice
S. Siljärvi, S. Pöysti, R. Toivonen, A. Hänninen1; 1Institute of Biomedicine, Turku, Finland; 2Turku University Hospital, Hospital District of Southwest Finland, Turku, Finland.

Akermansia muciniphila is a common symbiont in healthy gut of both humans and mice, while Citrobacter rodentium is a mouse pathobiont which promotes colitis in mice. Since gut microbiota is implicated in type 1 diabetes (T1D), the effects of these microbes were tested on diabetes and autoimmunity in the non-obese diabetic (NOD) mouse. While A. muciniphila colonization of C57BL/6J mice was safe without any inflammatory symptoms, C. rodentium infection in NOD mice induced dysbiosis. We found that dysbiosis had a more severe effect on pDC-depleted mice when compared to wild type mice. Deficiency of pDC during dysbiosis caused 20% weight loss when no change in wild type mice was seen. Colon epithelium was damaged, epithelial stress genes were upregulated and overall colon length was shorter in pDC-depleted mice. T cell analysis showed a lack of colon pDCs during dysbiosis blocks the induction of Foxp3+ regulatory T cells in coMLN and increases IFNγ production by both CD4 (T helper) and CD8 (T cytotoxic) cells. Our results indicate that pDCs have regulatory functions and in conjunction with Treg cells they control inflammation in the gut during dysbiosis.

P.D1.02.19 A potential link between age-related changes in the gut microbiome and changes in the germinal centre response of Peyer’s patches
M. Stebegg, S. Innocent, C. Gilbert, M. Linterman; Babraham Institute, Cambridge, United Kingdom.

The germinal centre (GC) response generates memory B cells and long-lived plasma cells that secrete high-affinity antibodies. In the gut, the GC response in Peyer’s patches (PPH) is an important source of IgA-secreting plasma cells. IgA antibodies are secreted into the gut lumen where they have an important role in controlling the composition of the gut microbiome. In ageing, the GC response in lymph nodes has been shown to be impaired, leading to reduced antibody affinity maturation and memory responses. We hypothesised that an impaired GC response in PPs affects the affinity and quantity of secreted IgA antibodies and thereby drives some of the age-related changes in the gut microbiome. When analysing the GC response of Peyer’s patches from aged C57BL/6 and Balb/c mice, we observed an age-related reduction in the proportion of GC B cells in 2 year old animals compared to 3 months old mice. Further, 16S-seq data from our ageing mouse colony confirmed changes in the gut microbiome of 2-year-old mice when compared to 3-month-old mice. While bacterial diversity was not affected, we observed a higher prevalence of Bifidobacteriales in young mice, while the old microbiome was enriched for Enterobacteriales. Taken together, there is a correlation between a poor GC response and changes in the microbiome in ageing. Future work aims to determine if this is a causal relationship.

P.D1.02.20 The effect of E. coli O83:B24:H34 on human and murine dendritic cells
L. Šukienková, P. Petřsková, J. Hrdý; Immunology and Microbiology, Prague, Czech Republic.

A balanced microbiome is greatly beneficial for a host. Any disturbance is associated with various problems and diseases, therefore, a number of experiments focus on correction for dysbiosis by probiotic supplementation. One of the promising probiotics appears to be Escherichia coli O83:B24:H34 (E. coli O83) but the exact mechanism of its positive effect has not yet described. To uncover it, we focused on differences between human and murine dendritic cells (DCs) after E. coli O83 stimulation. Both human and murine DC-like cells were obtained by in vitro differentiation from progenitor cells and then stimulated with probiotics and LPS for 24 hours. The appearance of DC surface markers was analysed by flow cytometry and the gene expression (qPCR) and secretion (ELISA) of various cytokines and enzymes were tested. The expression of DC activation markers CD80, CD86 and MHCII was significantly increased after stimulation by E. coli O83, indicating that DC-like cells are able to engulf and process probiotic antigens and reflecting their maturation state. Besides that, primed human DC-like cells produce higher amounts of IL-10 cytokine and express indole 2,3-di oxygenase enzyme. A slightly different mechanism occurs in the murine cells expressing also inducible nitric oxide synthase, typical for activated macrophages.

Together, results suggest that the positive effect of E. coli O83 is mediated by the reinforcement of tolerogenic DCs, thus supporting T regulatory cells which play a critical role in the induction of tolerance to self antigens and also to components of the microbiome.

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POSTER PRESENTATIONS

P.D1.02.21
Proinflammatory microbiota profiles associated with respiratory infections in older adults
S. Fuentes1, J. Ferreira1, J. Penning1, G. den Hartog1, J. van Beek1, G. van Boarier1,2
1National Institute for Public Health and the Environment, Biltoven, Netherlands, 2University medical Center Utrecht, Utrecht, Netherlands.

There is increasing evidence that interactions between the gut microbiome and the immune system can shape immune responses. With ageing, the immune response deteriorates leading to increased susceptibility to respiratory infections, posing a major health threat.

We studied the impact of gut microbiota composition on the development of respiratory infections using a cohort of older adults (>60 years) followed for influenza-like illness (ILI).

Participants were sampled within 48-72 hours of reporting an ILI (n = 238), 7-9 weeks after ILI (n = 201) and compared to individuals without any ILI event (n = 189).

Significant differences were found in the phyla Proteobacteria, Bacteroidetes and Firmicutes. Prediction analyses by Random Forest indicated the species Ruminococcus torques as top predictor for ILI. Stratification of groups by presence (R+) or absence (R-) of R.torques revealed higher abundance of pathogens from the E.coli/Shigella group. Furthermore, individuals from the R+ group showed significant lower diversity, a biomarker for gut microbiota resilience. Although levels of R.torques decrease after ILI recovery, levels of E.coli/Shigella remain elevated and beneficial bacterial groups (E.Akkermansia) remain reduced. As R.torques has been associated with a proinflammatory state, we measured inflammatory mediators including CRP, cytokines and chemokines. Redundancy analysis showed a proinflammatory profile in the ILI+R+ group both locally and systemically.

In conclusion, we reveal a proinflammatory microbiota profile in individuals presenting with ILI and identify potential microbial biomarkers for severity of disease. Understanding how gut microbiota influence onset and severity of respiratory infections can provide new leads for interventions targeted at microbiota modulation in the aging population.

P.D1.02.22
ROLE OF MICROBES IN HUMAN HEALTH, EFFECT ON INFECTIOUS DISEASE DYNAMICS
M. A. Yusuf
Gombe State Polytechnic, Iree, Nigeria.

The outbreak infectious disease in human health can now be investigated to identify microbes or pathogen and carriers for control of infections. The process of this microbiome outbreak can be in stages which can be blocked by different defense mechanisms: host is exposed to infectious particle by an infected individual, the mode of transmission and stability of an infectious person outside the host determine its infectivity. Some microbes show human immunodeficiency virus (HIV) are spread only by the exchange of bodily fluids, early contact of the microbes with a new host occur through an epithelial surface; the skin or the internal mucosal surface of the respiratory, gastrointestinal and urogenital tracts. The gut microbiota in disease-inflammation/microbial resident of the human gut are a major attributes in the development and maintenance of health but it differs from patient to patient. The causative agent fall into these: viruses, bacteria, fungi and protozoa. These approaches are also transforming our understanding of how interaction and focus between the gut microbiota and the host in order to provide an overview of the microbial role in basic biological processes and in the development and progression of major human diseases such as infectious disease, gastrointestinal cancer. The purpose of this study is to check the role of microbes in health and disease and how the research can be applied to medicine and therapeutic target in clinical practice.

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P.D1.03.01
The effector phenotype of human mucosal-associated invariant T (MAIT) cells in age-associated Clostridium difficile infections
I. Bernoll1, B. Bulitta1, L. Gräbe1, M. Neumann-Schaal1, J. Hofmann1, D. John1, A. Canbay1, D. Bruder1,2, L. Jänsch2,3
1Otto-von-Guericke University of Magdeburg, Magdeburg, Germany, 2Helmholtz Centre for Infection Research, Braunschweig, Germany, 3Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, 4Technical University Braunschweig, Braunschweig, Germany.

Clostridium difficile infection (CDI) can cause life-threatening inflammatory responses in the intestinal mucosa and has become the nosocomial infection with the highest medical and economical relevance in Germany. However, our knowledge about host immunity in human CDI is fragmentary, and especially the role of memory effector T cells, like mucosal-associated invariant T (MAIT) cells, remains elusive. MAIT cells are innate pathogen-reactive and restricted by MHC class I-related protein 1 (MR1), which presents antigenic bacterial metabolites of the riboflavin pathway. Presently the role of MAIT cells in CDI is unknown but interestingly their blood frequency decreases in age whereby the incidence of CDI increases. Thus, MAIT cells might mediate underscored protection against CDI infection and their functional impairment might increase susceptibility to CDI. We have recently reported the molecular phenotype of MAIT cells in healthy individuals (Bulitta et al., 2018) and here now elucidate their status and responsiveness in age-matched donor- and CDI-patient cohorts. As part of two consortia (ABINEP, CDIFF) and in cooperation with our clinicians we established an analytical pipeline allowing detailed molecular phenotyping with the support of proteomics. We will present the role of MAIT cells on the age-dependent alteration of MAIT cell effector functions, thereby complementing knowledge on the development and immunosenescence of the MAIT cell compartment. Beyond this we will present data characterizing the responsiveness of MAIT cells towards C. difficile. We defined the riboflavin synthesis of selected clinical isolates and using ex vivo isolated MAIT cells characterize which effector functions are activated dose- and MR1-dependently by C. difficile.

P.D1.03.02
Transcriptome profiling of Staphylococci-infected keratinocytes provides insight into the commensal-induced protective effect against S. aureus
K. Bütcher1, L. Klink1, B. Krismer1, A. Peschel1, B. Schütte1
1Department of Dermatology, University Hospital Tübingen, Tübingen, Germany, 2Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, University of Tübingen, Tübingen, Germany.

Introduction: Our skin is constantly exposed to a large number of pathogens while at the same time undergoing selective colonization by harmless commensal microorganisms such as S. epidermidis. We previously showed that secreted factors of S. epidermidis protect human and mouse skin against S. aureus infection. This work aims at elucidating the mechanism of this commensal-induced protective effect as well as providing a deeper understanding of how keratinocytes discriminate commensals from pathogens.

Materials and Methods: In order to identify the differential immune response triggered by commensals or pathogens we compared the transcriptomes of S. epidermidis and S. aureus-infected primary human keratinocytes by applying RNAseq. Furthermore, an in vitro skin infection model with keratinocytes and human skin explants as well as an in vivo epiputaneous mouse skin infection model were used to analyze the innate immune response induced by Staphylococci.

Results: We show that S. epidermidis is able to alarm keratinocytes by inducing the expression of NF-kappaB target genes while at the same time preventing excessive inflammation. Hereby, the alarm Interleukin-1, which itself is sufficient to induce the protective effect, seems to play a key role. Consequently, the S. epidermidis-mediated protection is lost in IL-1R-deficient mice.

Conclusion: In healthy skin S. epidermidis, as part of the skin microbiota, alarms keratinocytes and thus creates a protective environment which prevents S. aureus from colonizing the skin. Further studies will provide deeper insight into the mechanisms of the IL-1R-mediated modulation of the innate immune response, which reduces S. aureus skin infection.

P.D1.03.03
Synergistic action of soluble-pattern recognition molecule pentraxin 3 (PTX3) with myeloperoxidase (MPO)-mediated bacteria killing
K. Doiga1,2, D. Morone1, S. Valentin1, M. Sirion1, F. Petron1, A. Doni3, A. Infurato1, B. Battall1, A. Mantovani4
1Nippon Medical School, Kanagawa, Japan, 2IRCCS Humanitas Research Hospital, Milan, Italy, 3The University of Tokyo, Tokyo, Japan.

Introduction: PTX3 is a soluble-pattern recognition molecule that plays non-redundant protective roles against infections through pathogens recognition, complement regulation and opsonization. PTX3 comprises eight-identical protomers, each consisting of a conserved C-terminal pentraxin domain and an N-terminal domain unrelated to other proteins. We previously reported that PTX3 interacts with MPO, a bactericidal enzyme in neutrophils. Both PTX3 and MPO have been reported as protective molecules against Aspergillus fumigatus infection. Aim of our study is to investigate the role of PTX-MPO interaction in the bacterial killing. Results: Characterizing structural features of PTX3-MPO interaction, we found that PTX3-N terminal domain was responsible for MPO binding. Surprisingly, MPO enzymatic activity was increased in the presence of PTX3, an effect recapitulated by the PTX3-N terminal domain. Consistent with the effect, PTX3 enhanced MPO-mediated conidia killing and this action was mainly exerted by PTX3-N terminal domain. In contrast, when we evaluate the influence of PTX3 to conidia-bound MPO, we observed that full-length of PTX3 had no effect, while PTX3-N terminal domain maintains the capability to enhance both enzymatic and conidia killing activity of MPO. Finally, preliminary data indicated that exogenous PTX3 can amplify MPO-mediated conidia killing in neutrophil extracellular traps (NETs) from human neutrophils. Conclusions: Our data show that PTX3, through its interaction with MPO, can amplify MPO-mediated conidia killing mainly through the N-terminal domain. This could represent a novel antimicrobial mechanism of PTX3. Further studies will be needed to define the impact of our observations on the anti-microbial effects exerted by PTX3 in neutrophils.

438
Abstracts of the 5th European Congress of Immunology - ECI 2018 - The Netherlands
**POSTER PRESENTATIONS**

**P.D1.03.04**
Effects of silicon-rich water intake during chronic ingestion of aluminum on the systemic and peritoneal inflammation


1University of Niš, Medical Faculty, Niš, Serbia, 2European University, Novi Sad, Serbia, 3University of Niš, Faculty of Occupational Safety, Niš, Serbia, 4University of Belgrade, Faculty of Biology, Belgrade, Serbia.

Introduction: Ingestion of the aluminum by nutrients lead to its accumulation in human tissues, inducing various disorders. Silicon-rich water is a great source of bioavailable silicic acid, a natural antagonist of aluminum. The aim of study was to evaluate the role of silicon-rich water intake on the systemic inflammation and functional characteristics of neutrophils in mice. Materials and Methods: Two-month-old female Wistar rats were divided into three groups: control, aluminum chloride treated (2.5 mg/kg body weight in 0.5 mL of distilled water) and aluminum chloride treated group subjected to silicon-rich water (15 mg/L). Control rats underwent sham gavage and received standard or aluminum-rich water (n=7/group). Body weight and rectal temperature were measured at the beginning of the experiment and after 10 days. Results: Significant differences in the body weight gain were observed between the control and the aluminum chloride treated group. At the end of the experiment, the severity of colitis and colonic inflammation was evaluated. Conclusions: Silicon-rich water intake significantly improved the body weight gain of rats with colitis. Silicon-rich water intake decreased the severity of colitis and colonic inflammation.

**P.D1.03.05**
High protein diet influences the severity of colitis in mice


Institute of Microbiology of the CAS, Prague, Czech Republic.

Introduction: Gut microbiome composition is strongly influenced by our diet, which also influences immune response. The aim of our investigation was to examine the effects of diet, microbiome and immune response with inflammatory bowel disease development. Methods: We kept conventionally (CV) reared and germ-free (GF) BALB/c mice and RAG2/-/- mice on BALB/c background on High protein diet (HPD) or Control diet (CTRL) for 2-3 weeks. Then, we used 3% Dextrane Sodium Sulfate in drinking water to induce acute colitis. Results: We determined the severity of colitis in BALB/c mice by ELISA. HPD-fed mice showed lower levels of TNF-α in feces of CV mice compared to CTRL. HPD-fed mice had shorter colon, higher disease activity index (DDAI) and higher weight loss. Histological examination of colon samples showed more damage of the tissue. Conclusions: Inflammatory colitis was more severe in mice fed with HPD. Although there were no major differences in the microbiome composition of HPD-fed mice versus CTRL, we identified potential shifts in gut microbiota composition with HPD intake. Our data suggests that HPD fed mice are more susceptible to colitis.

**P.D1.03.06**
Deciphering the microbiome and metabolic factors contributing to protection against ulcerative colitis


1Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, 2ICVS/3B’s – PT Government Associate Laboratory, Braga/Guimarães, Portugal, 3Departamento de Genómica y Salud, Centro Superior de Investigación en Salud Pública – FISABIO, Valencia, Spain, 4Centers of Biomedical Research Network (CIBER) in Epidemiology and Public Health, Madrid, Spain, 5Instituto de Tecnologia Química e Biológica (ITQB) António Xavier, Universidade NOVA, Oeiras, Portugal, 6Molecular Oncology Research Center, Bariatric, Cancer Hospital, São Paulo, Brazil, 7ISS – Instituto de Investigação e Inovação em Saúde, Universidade de Porto, Porto, Portugal, 8IMIC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal.

Introduction: Intestinal bowel disease is an immune-mediated disorder triggered by environmental factors affecting the mucosal barrier and the gut microbiota balance. While analyzing DSS-induced colitis in genetically similar C57BL/6 mice housed in two different animal facilities, we identified a group of animals with a remarkable resistance to disease development. We aim to identify the microbial organisms/metabolites responsible for the protective phenotype.

Methods: The two groups of mice display distinct microbiome and metabolic profiles, clustering separately in multivariate data analysis. We identified 2 bacterial species that are statistically enriched in resistant mice. These bacteria can be used to selectively cultivate fecal microbiota transplant from resistant mice to susceptible mice that are likely to develop the disease.

**P.D1.03.07**
L-Threonine supplementation during colitis induction impairs goblet cell number and delays disease recovery


1Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal, 2ICVS/3B’s – PT Government Associate Laboratory, Braga/Guimarães, Portugal, 3Instituto de Tecnologia Química e Biológica (ITQB) António Xavier, Universidade NOVA, Oeiras, Portugal, 4INFACTS – Instituto of Research and Advanced Training in Health Sciences and Technologies, Department of Sciences, University Institute of Health Sciences (UICS), CESPU, CRL, Gandara, Portugal, 5UBIO, REQUIME, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal, 6Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal, 7ISS – Instituto de Investigación e Inovação em Saúde, Universidade do Porto, Porto, Portugal, 8IMIC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal.

Introduction: Dietary nutrients have emerged as potential therapeutic adjuncts for inflammatory bowel disease (IBD) given their impact on intestinal homeostasis through the modulation of immune response, gut microbiota composition and epithelial barrier stability. Several nutrients have already been associated with a protective phenotype.

Methods: The two groups of mice display distinct microbiome and metabolic profiles, clustering separately in multivariate data analysis. We identified 2 bacterial species that are statistically enriched in resistant mice. These bacteria can be used to selectively cultivate fecal microbiota transplant from resistant mice to susceptible mice that are likely to develop the disease.

Results: The two groups of mice display distinct microbiome and metabolic profiles, clustering separately in multivariate data analysis. We identified 2 bacterial species that are statistically enriched in resistant mice. These bacteria can be used to selectively cultivate fecal microbiota transplant from resistant mice to susceptible mice that are likely to develop the disease.

Conclusions: These results suggest that supplementation with threonine during colitis induction impairs goblet cell number and delays disease recovery. This reinforces the importance of a deeper understanding regarding threonine supplementation in IBD.

**P.D1.03.08**
Comparative genomics and in silico epitope prediction for the development of specific monoclonal antibodies for microorganisms in drinking water

M. Götthä, S. Happe, M. von Nickisch-Rosenegk, K. Hanisch

1University Potzdam, Department of Biochemistry and Biology, Immunotechnology group, Potsdam, Germany, 2Fraunhofer Institute for Cell Therapy and Immunology, Branch Biometrics and Bioanalytics and BioProcesses (IZI-BB), Department of Biometrics and Biosensors, Potsdam, Germany.

Introduction: Contamination of drinking water by pathogenic microorganisms is a great concern worldwide and leads to severe infections in humans. So far, the diagnostic detection of these pathogens is mainly performed by microbiological methods such as cultivating the sample on agar plates and counting of the colonies. Flow cytometry and cell-enzyme-linked immunosorbent assay (ELISA) are alternative methods to improve the monitoring of water quality. Therefore, specific monoclonal antibodies are indispensable for these techniques.
Methods: In this study, we performed a comparative genomics analysis based on next generation sequencing data of 50 microbial genomes to identify E.coli specific antigens useful as potential antibody targets. The genes presented in 30 different pathogenic E.coli strains but absent in 20 nonpathogenic E. coli/non-E.coli strains were subjected to in silico analysis to identify secreted or surface-expressed proteins.

Results: From 226.735 genes we obtained a total of 10 genes which encode potential protein candidates. We analyzed the resulting proteins for linear epitope prediction as well as antigenic properties. The epitopes with the highest scores to harbor specific epitopes have been cloned into a viral carrier protein used for immunization. We then used the resulting recombinant monoclonal antibodies specific to pathogenic E.coli, which could be part of a diagnostic tool for the detection of drinking water contaminations.

Conclusions: The described bioinformatic approach is able to identify novel epitope candidates for the generation of specific monoclonal antibodies specific to pathogenic microorganisms.

P.D1.03.09

Innate Recognition and Inflammammasome Activation in Human Myeloid Immune Cells by Methanogenic Archaea

T. Vierbuchel, C. Bang, R. Schmitz, H. Heine

1Research Center Borstel, Div. of Innate Immunity, Borstel, Germany, 2University Hospital Schleswig-Holstein, Institute of Clinical Molecular Biology (IKMB), Kiel, Germany, 3Kiel University, Institute for General Microbiology, Kiel, Germany.

The importance of the microbiota on health and immune homeostasis is widely accepted and the interaction between the microbiota and our body is currently investigated. However, most studies of this research are focused on bacteria alone, although viruses, fungi and archaea are also part of this microbial community. Recent studies showed that archaea are present at nearly every part of the body but their contribution to health and disease is not understood. It is known that the gut-associated methanogenic archaeon Methanospiruromonadaceae induces inflammatory responses, however, the mechanism how this archaeon is sensed by the immune system has not been evaluated until now. This study aims to elucidate the receptors, archaean structures and signaling pathways that are engaged upon activation of human immune cells by M. stadtmanae. We show that M. stadtmanae induces secretion of pro-inflammatory cytokines as well as expression of type I and III interferons in primary human myeloid cells as well as in the monocytic cell line BLAER1. CRISP5/Cas9 generated KO cells were used to identify TLR7 and TLR8 as main receptors for recognition of M. stadtmanae. Furthermore, the archaean induces a TLR9-dependent activation of the NLRP3 inflammasome sharing features with the LPS-induced alternative pathway.

P.D1.03.10

The antigen specificity of resident memory T cells in the human lungs

A. Oja, F. Morgana, B. Bardoe, D. van der Zwan, J. Mok, M. Moos, G. Brasser, R. van Lier, W. van Esch, P. Hombrink

1Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, 2Bacterial Infections and Immunity, University Medical Center Utrecht, University of Utrecht, 3Department of Reparative and Inflammatory Medicine, Amsterdam, Netherlands.

Resident memory T-cells (TRM) are crucial for local adaptive immune responses. In the lungs TRM are elementary for protection against airborne pathogens. This protection is superior relative to that provided by circulating T-cells and is modulated by the release of cytotoxic molecules. As TRM are in discrepancy with circulation and most antigen discovery studies rely on peripheral blood for readout, little is known about the magnitude, phenotype and specificity of protective T-cell populations in the lungs. In this study we simultaneously dissect the phenotype and function of CD4+ and CD8+ T-cells in human lung tissue and compare these to the blood compartment. By using extensive HLA-typed and combinatorial coding screens we analysed the phenotype and frequency of lung CD8+ T-cells specific for respiratory and systemic viruses in donors with no recent history of infection. We demonstrate an enrichment of respiratory specific T-cell subsets in the lung CD8+ T-cell compartment. While influenza and RSV reactive CD8+ T-cells are biased to a CD103+ T RM phenotype, those specific for CMV and EBV are phenotypically identical to circulating cells. In contrast, influenza reactive CD4+ T-cells in the lungs lack expression of CD103, suggesting a strict spatial regulation. In addition we mapped the lung CD4+ T-cell response to the human pathogens Staphylococcus aureus and pneumonia using libraries of recombinant proteins and discovered novel immunodominant responses. The ability to monitor antigen-specific T-cell responses in human lungs, will facilitate subsequent transition into vaccination strategies that aim to boost local protection to airborne pathogens.

P.D1.03.11

MAIT cells are recruited to the intervillos space of the placenta by placlental-derived chemokines


1Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, 2Department of CLINTEC, Karolinska Institutet, Stockholm, Sweden, 3Center for Fetal Medicine, Karolinska University Hospital, Stockholm, Sweden, 4Department of Women’s and Children’s Health, Karolinska Institutet, Stockholm, Sweden, 5Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden, 6Program in Emerging Infectious Diseases, Duke-National University of Singapore Medical School, Singapore, Singapore, 7Department of Oncology/Pathology, Karolinska Institutet, Stockholm, Sweden, 8Rheumatology and Inflammation Research, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, 9Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden.

The intervillos space of the placenta is a part of the fetal-maternal interface, where maternal blood enters to provide nutrients and gas exchange. Little is known about the maternal immune cells at this site, which are in direct contact with fetal tissues. We have characterized the immune cell composition and chemokine profile in paired intervillos and maternal chorionic villi from healthy mothers giving birth at term. We found that mucosal-associated invariant T (MAIT) cells were enriched in the intervillos space compared to circulating T-cells and is modulated by the release of cytotoxic molecules. As TRM are in discrepancy with circulation and most antigen discovery studies rely on peripheral blood for readout, little is known about the magnitude, phenotype and specificity of protective T-cell populations in the lungs. In this study we simultaneously dissect the phenotype and function of CD4+ and CD8+ T-cells in human lung tissue and compare these to the blood compartment. By using extensive HLA-typed and combinatorial coding screens we analysed the phenotype and frequency of lung CD8+ T-cells specific for respiratory and systemic viruses in donors with no recent history of infection. We demonstrate an enrichment of respiratory specific T-cell subsets in the lung CD8+ T-cell compartment. While influenza and RSV reactive CD8+ T-cells are biased to a CD103+ T RM phenotype, those specific for CMV and EBV are phenotypically identical to circulating cells. In contrast, influenza reactive CD4+ T-cells in the lungs lack expression of CD103, suggesting a strict spatial regulation. In addition we mapped the lung CD4+ T-cell response to the human pathogens Staphylococcus aureus and pneumonia using libraries of recombinant proteins and discovered novel immunodominant responses. The ability to monitor antigen-specific T-cell responses in human lungs, will facilitate subsequent transition into vaccination strategies that aim to boost local protection to airborne pathogens.

P.D1.03.12

Experimental high-fat diet consumption leads to intestinal barrier damage and severe colitis

K. Klimesova, N. Galanova, A. Fajstova, S. Coufal, M. Kostovcik, M. Buganova, H. Taslikova-Hogenova, M. Kverka; Institute of Microbiology CAS, Prague, Czech Republic.

It is important environmental factor influencing gut homeostasis. Increased fat intake is related to higher risk of inflammatory bowel disease development. Here, we focused on effects of high-fat diet consumption on colitis development in mouse model. Fourteen days before colitis induction, we transferred mice to diet with increased or normal fat content - high-fat diet (HFD, 22% fat) or control diet (CD, 5% fat), respectively. Then, we induced colitis with 3% dextran sodium sulfate (DSS) in drinking water for 7 days or we used DSS treatment for 4 weeks followed by 3 weeks recovery. Mice consume also significantly longer colon and bigger spleen. Moreover, HFD mice showed increased permeability of intestine measured by fluorescein isothiocyanate-conjugated dextran and mild inflammatory changes observed by microscopic analysis of hematoxylin/eosin stained sections. DSS treatment in HFD mice led to significant reduction of their body weight, diarrhea and rectal bleeding. The length of colon did not change significantly but spleen weight doubled compared to CD mice. In spleen of HFD mice, we found significant increase in Th17 cells - CD4+RORgT + population measured by flow cytometry, and higher production of IL-17A in the presence of IL-6 and IL-23. In conclusion, we confirmed that HFD significantly increased the sensitivity of mice to DSS colitis. Mice consuming HFD developed low local and systemic inflammation which could lead to mucosal immune system exhaustion and induction of systemic Th17 response. The study has been supported by Czech Science Foundation grants no. 16-06325S and 17-06632Y.

P.D1.03.13

Polysaturated fatty acids (PUFAs) dampen the inflammatory antiviral immune response by diminishing two dimensional (2D) antigen recognition by T cells

E. M. Kolaowle, B. D. Evavold;

University of Utah, Salt Lake City, United States.

Polysaturated fatty acids (PUFAs) have been shown to dramatically influence inflammatory responses. Although omega-3 fatty acids lead to the production of less inflammatory metabolites, little is known about how PUFAs change surface protein interactions and T cell activation. To probe the protein interactions, we utilized novel 2D based technologies for single cell and single molecule analyses of T cell receptor (TCR) engagement with peptide-MHC (pMHC) antigens. We found that dietary fish oil decreased 2D affinity of T cell receptor transgenic and polyclonal T cell responses in both CD8 and CD4 T cells at peak anti-viral immunity to lymphocytic choriomeningitis (LCMV) infection. In addition, an omega-3 based diet increased structural lipid content in plasma membranes leading to a reduced T cell receptor frequency and changes in the prevalence of immunodominant epitopes. This reduced affinity led to decreased markers of activation and effector function. These data indicate that a major effect of dietary omega-3 fatty acids on T cells is the modification of the cell membrane to dampen the initial recognition of antigen.
Introduction: Ulcerative Colitis (UC) is a relapsing disorder of the gastrointestinal tract characterized by intestinal inflammation and epithelial injury. Although the precise cause of UC remains unknown, microbial-host interactions and mitochondrial dysfunction play a critical function. Therefore, it is needed to know the role that a natural negative regulator of mitochondrial respiration such as Methylation-controlled J protein (MCJ) exerts in the disease. UC remains unknown, microbial-host interactions and mitochondrial dysfunction play a critical function. Therefore, it is needed to know the role that a natural negative regulator of mitochondrial respiration such as Methylation-controlled J protein (MCJ) exerts in the disease. In this study we examined the effect of some of these food additives on gut inflammation. Maltodextrin (MDX)-enriched diet exacerbated intestinal inflammation in experimental models of colitis and ileitis. Analysis of the mechanisms underlying the detrimental effect of MDX revealed up-regulation of the inositol-requiring enzyme (IRE)1β, a sensor of endoplasmic reticulum (ER) stress, in goblet cells and reduction of mucin-2 expression with no significant change in mucosa-associated microbiota composition. Stimulation of murine intestinal crypts and the human mucus-secreting cells with MDX induced IRE1β via a p38 MAP kinase-dependent mechanism. Treatment of mice with the ER stress inhibitor Tauroursodeoxycholic acid prevented mucin-2 depletion and attenuated colitis in MDX-fed mice. Interestingly, mice receiving a prolonged MDX-enriched diet exhibited low grade intestinal inflammation, which was characterized by focal inflammatory infiltrates, distortion of gland architecture and exines. In conclusion, this study shows, for the first time, that MDX-enriched diet triggers ER stress in goblet cells with consequent reduction of the intestinal content of mucin-2, thus making the host more sensitive to colitogenic stimuli. Our data supports the hypothesis that western diet rich in the food additive MDX can contribute to gut disease susceptibility.
**POSTER PRESENTATIONS**

**P.D1.03.19**

*Vancomycin-resistant T cells proliferate in response to phosphoantigens released from erythrocytes infected with axenial and gametocyte stage Plasmodium falciparum*

C. Liu, N. Emami1, J. Pettersson1, L. Ranford-Cartwright1, I. Forge, J. Parrymd1.

1Medical cell biology Uppsala university, Uppsala, Sweden, 2Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, 3Department of Chemistry, Uppsala University, Uppsala, Sweden, Institute of infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom.

**Introduction:** Vγ9Vδ2 T cells are the dominant γδ T cell subset in human peripheral blood. Vγ9Vδ2 T cells are activated by phosphoantigens, primarily isoprenoid pyrophosphates. It has been shown that phosphoantigens that stimulate Vγ9Vδ2 T cells are released from Plasmodium falciparum-infected erythrocytes upon schizont rupture. However, to date it was reported that release also occurs at the ring stage of the parasite life cycle. We therefore set out to investigate whether phosphoantigens are released at any other stage of the blood stages. *Basic Method:* Pure cultures of Plasmodium falciparum-infected erythrocytes at all asexual stages were obtained using a combination of a plasmin and sorbitol synchronization and of sexual gametocytes by using heparin. Sterile-filtered media from the cultures was assessed for its ability to cause proliferation of Vγ9Vδ2 T cells from human PBMCs. Results: Vγ9Vδ2 T cells were proliferated by addition of the media from erythrocytes infected with parasites at all blood stages (ring, trophozoite, schizont and rupturing schizont and gametocytes). The proliferation was caused by phosphoantigens, verified by phoshpatase-treatment. The iron-levels in the media from infected cultures were not elevated indicating that erythrocyte rupture was minimal. **Conclusion:** Phosphoantigens in sufficient quantities to stimulate Vγ9Vδ2 T proliferation are released by intact parasite-infected erythrocytes at all blood stages of the parasite life cycle. *Grants:* This work was supported by grants from INFRAVEC (EU/FP7) to IF and the Swedish Research Council, AFA Research Foundation and Claus Greshousinsky’s Foundation to IP.

**P.D1.03.20**

*Assessment of multivalent viral vectored vaccines against outbreak pathogens: Ebola, Marburg & Lassa*

A. Flaxman1, S. Sebastian1, C. Gilbridge1, H. Sharpe1, S. Dowall1, S. Charlton1, J. Purushothaman1, A. Hill1, S. Gilbert1, T. Lambe1; 1Jenner Institute, University of Oxford, Oxford, United Kingdom, 2Public Health England, Salisbury, United Kingdom.

Since the 2013-2016 Ebola outbreak, there have been a number of other documented outbreaks of lethal haemorrhagic fever caused by Filoviruses and Arenaviruses. Filovirus family members including EbolaVirus and Marburg virus and arenaviruses such as Lassa virus cause haemorrhagic fevers with high mortality rates in humans. As yet, no specific treatment or prophylactic multivalent vaccine against Filoviruses or Arenaviruses has been licenced. It is generally accepted that either a mixture of monovalent vaccines or, preferably, a multivalent vaccine, will be required to confer protective immunity against viral haemorrhagic fever. The costs of developing individual vaccines against Filoviruses and an arenavirus (Lassa virus [LASV]) may be prohibitively high. Considering the geographical overlap between Ebola, Marburg and Lassa virus endemic areas, a multivalent vaccine would be of significant benefit. Our pre-developed core vaccine platforms (adenoviral vectors and MVA) can express multiple antigens and have demonstrated capability for the induction of durable immune responses in other infectious disease settings e.g. influenza/malaria. We have now used this technology to develop multivalent vaccines against several lethal haemorrhagic fever-causing viruses, including Filoviruses [EbolaVirus & Marburg virus] and Arenaviruses (Lassa virus). These vectors elicited antigen expression in vitro and were immunogenic in vivo. We assessed different vaccine formulations in mice and tested efficacy by challenging guinea pigs with Ebola Zaire post-vaccination. Our viral vectored vaccines showed protection from disease in this model. Therefore, we have successfully developed preclinical vaccine candidates against three outbreak pathogens (Ebola, Marburg and Lassa) using a multivalent platform.

**P.D1.03.21**

*Complex and unusual antigen-specific activation of Vγ9Vδ2 gamma-delta T-cells by the BTN3A-receptor complex.*

G. A. Rhodes, S. Smith, J. Towrodale1, N. McCarthy1, M. Eberl1.

1University of Cambridge, Cambridge, United Kingdom, 2Centre for Immunobiology, Blizard Institute, London, United Kingdom, 3Systems Immunity Research Institute, Cardiff University, Cardiff, United Kingdom.

The gamma delta T-cell pool in human blood is dominated by Vγ9Vδ2 (Vδ2+) T-cells, which are specialised to detect phosphoantigens (pAg) produced by microbes and tumours. Activation of Vδ2+ T-cells by pAg signals the expression of BTN3A family molecules by presenting cells. At present it is not clear what form the expression of the three BTN3A isoforms transmits activation signals nor how they are regulated. To investigate this, we used BTN3A knockout HeLa cells (HeLa/sgBTN3A), generated by CRISPR/cas9 gene editing, together with stable re-expression of BTN3A isoforms in all combinations. BTN3A1 and BTN3A3 proteins were subject to cell intrinsic suppression, whereas BTN3A2 was expressed constitutively. In co-culture experiments with expanded Vγ9Vδ2 T-cells, we confirmed that BTN3A1 was necessary but not sufficient to transmit activation signals, with BTN3A2 and/or BTN3A3 also being required. There were differences in responses induced by activating stimuli, HMB-PP, IPP and antigen antibody CD277 20.1. The combination of BTN3A1 with BTN3A3 induced more potent cytotoxicity and cytokine production as measured by lactate dehydrogenase release and IFNγ secretion in co-culture medium, whereas the BTN3A2/BTN3A3 combination was non-functional for these stimuli. A critical role for the BTN3A1 and BTN3A2 (juxta-membrane domains, pivotal in shaping the BTN3A-dependent activating epitope required for Vδ T-cell receptor engagement, was shown. Our results reveal additional complexity and highlight the unusual nature of the antigen-specific activation of human Vδ2+ T-cells, which is regulated by cell intrinsic mechanisms linked to infection and cell transformation by pAg sensing and control of BTN3A protein stability.

**P.D1.04.01**

*Anaeroplasma of the phylum Tenericutes induce mucosal TGF-α and promote IgA production.*

A. Beller1, A. Kruglov1, P. Durek1, K. Werner1, V. van Goetech1, J. Ninnemann1, K. Lehmann1, B. Siegmund1, U. Hoffmann1, G. A. Heinz1, M. F. Mashreghi1, A. Radbruch1, H. D. Chang2.

1German Rheumatism Research Center (DRFZ), a Leibniz Institute, Berlin, Germany, 2Belazersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russian Federation, 3Charité - Universitätsmedizin Berlin, Medical Department I (Gastroenterology, Infectiology, and Rheumatology), Campus Benjamin Franklin, Berlin, Germany.

In humans and mice, mucosal immune responses are dominated by IgA antibodies and the cytokine transforming growth factor beta (TGF-β), suppressing unwanted immune reactions but also targeting immunoglobulin class switching to IgA. IgA plays a central role in the interplay between the host cells and microbiota at the mucosal surfaces. The production of IgA as such is controlled by microbiota and requires the cytokine “transforming growth factor beta” (TGF-β), which induces IgA switch transcripts. However it has not been clear which bacteria directly produce IgA and how they do it. It had been suggested that eosinophils promote the generation and maintenance of mucosal IgA-expressing plasma cells. Here we demonstrate that not eosinophils, but specific bacteria enhance mucosal IgA production. We also now show that not the colonization of the intestinal tract and the stimulation of the intestinal immune with bacteria as such, but rather distinct microbial species lead to the preferential induction of intestinal IgA. Our data indicate that the bacteria of the genus Anaeroplasma increase numbers of IgA secreting plasma cells in the lamina propria of the small intestine, and significantly enhance mucosal IgA levels. Anaeroplasma controls IgA expression by inducing expression of the IgA class switch-inducing cytokine TGF-β in Fc receptor-positive cells of Peyer’s patches. Anaeroplasma is also a part of the human microbiome. Its anti-inflammatory properties of inducing the immune regulatory cytokine TGF-β, strengthening the intestinal barrier by enhancing mucosal IgA, make it an interesting probiotic for the prevention and treatment of intestinal inflammation. **P.D1.04.02**

*Immune dysfunction in common variable immunodeficiency disorder: a role for Enteroceccus?*

R. Berbery, P. Elberbroek, J. van Montfrans, M. Rogers, M. Veenen, F. Paganelli, V. Dalim, M. van Hagen, A. van der Ven, J. van Loar, R. Willems, H. Leavis.

1University Medical Center Utrecht, Utrecht, Netherlands, 2Erasmus Medical Center, Rotterdam, Netherlands, 3University Medical Center Groningen, Groningen, Netherlands.

Common variable immunodeficiency disorder (CVID) is the most common primary immunodeficiency, but its etiology is not well understood. Currently, only 10% of cases can be explained by genetic variants. This humoral immunodeficiency is often accompanied by immune dysregulation phenomena, including autoimmunity, granulomas and lymphoproliferation. Better understanding of the cause of CVID and the immune dysregulation phenomena is key improving care for these patients. Given the limited genetic contribution to CVID and prevalence of gastrointestinal symptoms in these patients, a role for the gut microbiome has been hypothesized. We aimed to characterise the composition of the faecal microbiota of 106 CVID patients and 49 healthy controls (HC) cross-sectionally. 16S rDNA profiling revealed decreased diversity in CVID patients compared to HC. Interestingly, IgA deficiency did not change alpha diversity within CVID patients. Although patients did not cluster separately from controls in unsupervised analyses distinct groups of bacterial taxa were significantly abundant in CVID patients compared to HC. Enterococcus sp were increased in patients with immune dysregulation (n=50) as compared to those without (n=56), regardless of use of medication. Veillonella dispar was associated with enteritis, while bacteria belonging to the Clostridiales and Bifidobacteriales families were more abundant in the faeces of healthy patients. To conclude, immune dysregulation in CVID was associated with low bacterial diversity and increased abundance of Enterococcus, a group of bacteria that has recently been implicated in autoimmunity.
**Poster Presentations**

**P.D1.04.03**
**Understanding the interaction between vaginal microbiota and immunity; the role of immunoglobulin A**

A. Bredeweld, H. Schuster, L. Pedrò-Cas, R. Mebius, D. Budding, M. van Egmond,
VUmc, Amsterdam, Netherlands.

Immunoglobulin (Ig) A is the most prevalent antibody at mucosal surfaces and alterations in IgA coating of gut-resident bacteria have been associated with inflammatory bowel disease. An unbalanced vaginal microbiota was recently linked to preterm birth, raising the question whether IgA is involved in this process. Still, much less is known about the interaction between IgA, bacteria and the immune system of the female genital tract.

We determined the vaginal microbial composition of healthy women using 16S-pro. IgA coating of the vaginal microbiota from healthy donors was measured with flow cytometry. Additionally, the presence of specific IgA against common vaginal bacterial strains in serum was examined. Neutrophils, monocytes and monocyte-derived dendritic cells (moDCs) were stimulated with IgA-opsonized vaginal cell-free supernatant to measure phagocytic capacity. Cytokine production after 24 h stimulation of cells with IgA-opsonized vaginal bacteria will be measured using ELISA.

Healthy vaginal microbial composition was predominated with Lactobacillus spp. Vaginal swabs contained a high proportion of IgA-coated bacteria. Neutrophils, monocytes and moDCs showed increased phagocytic capacity for IgA-opsonized vaginal bacteria. These results demonstrate a mucosal and systemic IgA response against vaginal bacteria. Cytokine profile of innate immune cells after stimulation with IgA-opsonized bacteria will be established and bacterial cell sorting as well as molecular microbiota analysis will be performed to study taxa-specific IgA coating. We aim to use IgA coating of vaginal bacteria as predictor for preterm birth.

**P.D1.04.04**
**BGC vaccination in the childhood of women influences their placenobiomediating pregnancy: vertical transmission of mycobacterial l forms**


1Group of Biotechnology and Special Microbiology, Medical University, University Obstetrics and Gynecology Hospital "Mother House", Sofia, Bulgaria, 2Institute of Microbiology of the CAS, Prague, Czech Republic, 3Institute of Biology and Immunology of Reproduction "acad. K. Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria, 4Medical University, University Obstetrics and Gynecology Hospital "Mother House", Sofia, Bulgaria, 5Institute of Microbiology of the CAS, Prague, Czech Republic.

Introduction: It has been shown that maternal microbiota drives early postnatal innate immune development and prepares the newborn for host-microbial mutualism by vertical transfer of microbial molecules. Recent studies with human placenta found the presence of dynamic placental microbiome with specific metabolic functions, which effects on the developing fetus and neonate is still unknown. Since live mycobacterial l-forms could be found in the blood of BCG-vaccinated people, we aimed to investigate a possible trans-placental transfer of BCG l-forms from the blood of healthy BCG-vaccinated pregnant women to their neonates as well as the presence of specific gamma/delta T cell response in the placenobiota. Materials and methods: Sterile obtained samples from early pregnancy deciduas, term placentas, maternal blood and cord blood were examined by isolation and appropriate cultivation, electron microscopy, qPCR and FACS. Results: We proved that the majority of the samples of maternal blood, gestational tissues and cord blood of healthy women delivered by healthy BCG-vaccinated pregnant women were colonized with mycobacterial l-forms. The transfer of l-forms occurs early in gestation and the maternal decidua mediates the process of placenta colonization. No specific expansion of pathogen-reactive VδT gamma/delta T cells was detected. Conclusions: Novel data about mother-to-newborn transmission of mycobacterial l-forms suggests that BCG vaccination in the childhood of the woman may affect her placenobiome during pregnancy.

Acknowledgments: This study was funded by Bulgarian National Science Fund, project DN 03/5.

**P.D1.04.05**
**Effects of obesity on Tick-borne encephalitis (TBE) booster vaccination**


1Medical University of Vienna, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria, 2Dept. for Laboratory Medicine, Medical University Vienna, Austria, Vienna, Austria, 3Center of Virology, Medical University Vienna, Austria, Vienna, Austria, 4Center for Public Health, Medical University Vienna, Vienna, Austria.

Obesity has significantly increased worldwide and has, apart from related co-morbidities, direct effects on the immune system leading to immune dysfunction and increased susceptibility to infectious diseases. Thus, prophylaxis against vaccine preventable diseases is particularly important in obese individuals. In order to assess vaccine efficacy, we performed an open-label phase IV clinical trial with 37 obese people and 36 normal-weight controls, which were booster-vaccinated against tick-borne encephalitis (TBE). The general immunologic and metabolic profile along with vaccine-specific humorals and cellular immune responses were evaluated in sera and PBMC. Obese adults showed significantly increased metabolic (leptin, insulin, triglycerides, cholesterol) and pro-inflammatory (CRP) markers. Total immunoglobulin levels (IgM, IgA) were increased in obese vaccinees, while natural IgA against pathogens (PC) were significantly reduced, possibly indicating higher infection susceptibility. Obese individuals showed a stronger fold-increase of TBE-specific Ab titers 4 weeks after booster, which was positively correlated with metabolic parameters. However, Ab levels declined significantly faster within 6 months in this group. Also, higher inflammatory cytokines were detected in obese vaccinees before vaccination and distributions of B- and T-cell subsets differed between obese and control group. Vaccine reactogenicity (local and systemic) was higher in obese subjects. Our results indicate that TBE booster vaccination was effective at humoral and cellular level in obese individuals; however, the higher decline rate in obese might lead to shorter long-term protection. Whether the effects of obesity on primary TBE vaccination are similar remains to be investigated. *contributed equally by investigator initiated industrial funding (Pfizer)

**P.D1.04.06**
**Study on the effect of metformin and succinate on the differentiation and functions of mesenchymal stem cell**

H. Hao, B. Chiang.

1Graduate Institute of Oral Biology, School of Dentistry, Taipei, Taiwan, 2Graduate Institute of Clinical Medicine College of Medicine of National Taiwan University, Taipei, Taiwan.

Introduction: Mesenchymal stem cells (MSCs) have been used for a variety of diseases due to their unique properties. Metabolic regulation including metabolic modulators treatment and glucose supplementation has indicated to enhance MSCs properties. But the metabolic regulation to immunomodulatory function of MSCs remains unclear.

Hyperglycemia induces inflammation and impairs TCA cycle, causing immunity-related succinate accumulated in T2D, which can treat by MSCs and metformin. Combined mechanisms.

Results: we found that metformin and succinate could enhance suppressive function to T cell and also the differentiation capacity. The mRNA level of iNOS increases in treated-

Materials and Methods: MSCs are derived from the bone marrow of the 4-5 week old female BALB/c mice. Isolated-MSCs are treated by metformin or succinate to assay the

mechanisms.

Hyperglycemia induces inflammation and impairs TCA cycle, causing immunity-related succinate accumulated in T2D, which can treat by MSCs and metformin. Combined mechanisms.

Conclusions: Metformin- or succinate-treated MSCs demonstrated enhanced suppressive function and different metabolisms. We believe further clarification on the metabolic mechanisms involved in the development and functions of MSCs might shed light on future application of MSCs for immune modulation.

**P.D1.04.07**
**Metronidazol changes the susceptibility to iniquimod-induced skin inflammation**


1Institute of Microbiology of the CAS, Prague, Czech Republic, 2Graduate Institute of Oral Biology, School of Dentistry, Taipei, Taiwan, 3Dept. for Laboratory Medicine, Medical University Vienna, Austria, 4Dept. for Laboratory Medicine, Medical University Vienna, Austria, 5Institute of Microbiology of the CAS, Prague, Czech Republic, 6Dept. for Laboratory Medicine, Medical University Vienna, Austria, 7Institute of Microbiology of the CAS, Prague, Czech Republic.

Introduction: Alteration of microbiota influences the immune response of the host. Overall changes in microbiota composition are also involved in the psoriasis incidence and maintenance. The aim was to reveal whether perioral antibiotic treatment has the potential to mitigate experimental skin inflammation and to describe in detail subsequent changes in microbiota composition on the skin and in the intestine. Methods: To alter the microbiota, we gavaged BALB/c mice with antibiotics, specifically metronidazole (MET), vancomycin, colistin and streptomycin, and their mixture (ABTs) or water (W) in controls consecutively for 21 days. To induce the iniquimod-induced skin inflammation (IISI), we applied iniquimod on the shaved back skin for last 6 days. Then, we evaluated the severity of IISI. We used Illumina approach to assess the changes in microbiota. Results: Compared to W control mice, we found that MET and ABTs mitigated the severity of IISI in all clinical parameters. This effect was associated with downregulation of Th17 response. Next, we found that treatment with MET and ABTs significantly decreased the intestinal microbial diversity but microbial diversity on skin was significantly changed only after treatment. Although ABTs increased the abundance of Lactobacillales, other members, such as Clostridiales and Bacillales, were decreased. Conclusions: Our findings suggest that differences in microbiota composition can influence experimental skin inflammation development and can lead to identification of specific microbial patterns associated with the disease. This study was supported by Ministry of Health of the Czech Republic grant nr. 1S-30782A

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 443
metabolic reprogramming in these cells. Furthermore, pentanoate strongly enhanced IL-10 production in regulatory B cells (Bregs) and Th17 cells by reprogramming their metabolic activity. This effect was dependent of gene expression by exhibiting strong histone deacetylase (HDAC)-inhibitory activity, thereby reducing IL-17A production and suppressing pathogenic Th17 responses mechanisms. While a potent immunomodulatory activity for acetate, propionate and butyrate has been described in various experimental models, the role for the SCFAs Short-chain fatty acids (SCFAs) are able to induce differentiation of regulatory T cells (Tregs), which is one of the crucial mechanisms by which commensal bacteria contribute to the Therefore, we characterized different mouse models addressing physiological bone homeostasis in naïve C57BL/6 and Rag1-/- mice of this study. Principle Coordinate Analysis (PCoA) of weighted and unweighted UniFrac distance matrices did not separate DEREG and wildtype mice gut microbiomes. PCOA clustered samples according to their sample identifier and age rather than according to the different time points before and after Treg depletion. Conclusion: In this work we analysed the gut microbiome variation associated with Treg depletion in mice over time. Intramicrobiome variability of the gut microbiome over time was less pronounced compared to inter-microbiome variability before Treg depletion.

Roles of IRF-1 in negative cross-talk between Liver X Receptors (LXRs) and IFN-γ signalling

N. A. Letelier1, A. Lepetit1, A. M. Planas1, A. F. Valledor2
1Nuclear Receptor Group, Department of Cell Biology, Physiology and Immunology, School of Biology, University of Barcelona, Barcelona, Spain, 2Centre de Recherche INSERM UMC1231, F-21000, Dijon, France, 3Department of Brain Ischaemia and Neurodegeneration, Institute for Biomedical Research of Barcelona (IIBB), Consejo Superior de Investigaciones Científicas (CSIC), Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

Lever X Receptors (LXRs) are members of the nuclear receptor family of transcription factors that regulate lipid and glucose homeostasis and exert several roles at the interface between metabolism and the immune system. Pharmacological LXR activation induces the expression of genes involved in cholesterol efflux (e.g. Abca1 and Abcg1) and inhibition of cholesterol uptake [50]. Recent work from our group has revealed that LXR activation also induces the expression of the enzyme CD38 in macrophages, a transmembrane glycoprotein that controls NAD+ levels in tissues. Further, we have previously reported reciprocal cross-talk between IFN-γ signalling and the LXR pathway in macrophages. IFN-γ involves activation of the Jak-STAT1 pathway to promote the expression of primary IFN response genes, including the transcriptional regulator IFN regulatory factor-1 (IRF-1). To characterize the roles of IRF-1 in the cross-talk between IFN-γ and LXR activation, we compared the effects of IFN-γ stimulation on the expression of LXR target genes in macrophages from wild type and IRF-1 or STAT1 deficient mice. IFN-γ signalling resulted in reduced induction of Abca1 and Aim by LXR, effects that were STAT1-dependent but IRF-1-independent. In contrast, IFN-γ signalling and LXR cooperated in transcriptional induction of Cdx3 in a STAT1-dependent manner and this synergistic induction was drastically enhanced in IRF-1-deficient macrophages. These results suggest that IRF-1 serves to fine-tune the expression of CD38 during the macrophase response to IFN-γ. This work was supported by grant 201605.31 from Fundació La Marató de TV3 to A.F. Valledor; Nicole Letelier is a CONICYT fellowship awarded.

Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss

S. Lucas1, Y. Omatz1, J. Hofmann1, M. Böttcher1, A. Iljaváci2, K. Sarter1, O. Albrecht1, O. Schulze2, B. Krishnacoomur1, G. Krönke1, M. Herrmann1, D. Mougialakos3, T. Strowig1, G. Schett1, M. M. Zais1
1Department of Internal Medicine 3, Rheumatology and Immunology, Friedrich-Alexander-University Erlangen-Nürnberg (FAU) and Universitätsklinikum Erlangen, Erlangen, Germany, 2Department of Biochemistry, Developmental Biology and Molecular Pathology, Department of Internal Medicine 5, Medical School and Department of Pediatrics, Medical University of Lodz, Lodz, Poland, 3Department of Old Age Psychiatry and Psychotic Disorders, Lodz, Poland.

Background. Schizophrenia is a severe mental disorder characterized by firmly impaired thinking, emotions, and behaviors, affecting approximately 1% of the population worldwide. Increasing evidence suggests that, apart from neurochemical abnormalities, various immunological alterations are related to the pathogenesis of schizophrenia. Given a fact that Toll-like receptors (TLRs) play pivotal role in the initiation of innate immunity and inflammatory mechanisms in this study we determine the TLR expression in peripheral blood mononuclear cells (PBMCs) in schizophrenic patients.

Materials and methods. Twenty-seven adult European Caucasian patients with paranoid schizophrenia were included in this prospective study. Twenty-nine healthy volunteers were also randomly selected as a control group. PBMCs were isolated from whole blood by density gradient centrifugation. Total RNA was isolated from PBMCs by acid guanidinium thiocyanate-phenol-chloroform extraction. TLRs expression was evaluated using quantitative real-time polymerase chain reaction (qRT-PCR).

Results. We demonstrated that TLR1, TLR2, TLR4, TLR6, and TLR9 mRNAs expression were down-regulated in patients with schizophrenia in opposite to TLR3 and TLR7 mRNAs which manifested higher expression. TLR5 and TLR8 mRNAs demonstrated non-statistically significant alterations.

Conclusion. Altered expression of TLRs in PBMCs of schizophrenic patients may reflect an important interplay between TLRs and development of schizophrenia. Supported by the Medical University of Lodz (grant no 502/03/6-164-01/502-64-106).

Impact of Faecal3 Regulatory T cell depletion on the murine gut microbiome

C. Wilk1, D. Schöner2, A. Adamczyk1, E. Pastille1, A. Westendorf1, J. Bauer1, J. Richmann1
1Institute of Medical Microbiology, Essen, Germany.

Introduction: Besides the impact of the gut microbiota to shape the immune system in the intestine, the immune system itself is considered to shape the gut microbiota composition. The relevance of regulatory T cells (Tregs) in this context is unclear so far. We analyzed the gut microbiome in DEREG mice over a period of twenty days after depletion of Tregs by Diphtheria Toxin (DT).

Methods: We extracted DNA from stool samples of twenty-four mice (19 DEREG and 5 wild-type mice) at 5 different time points (day -7 [seven days before first DT application], day 0, 5, 10 and 20), derived from 3 independent experiments. Sequencing of the V3/V4 region of the 16S rRNA gene was performed using the Illumina MiSeq x 2 and 301 paired ends reads cartridge (Illumina). Data processing of the analysis was performed using the QIIME pipeline.

Results: Variation of the gut microbiome’s alpha and beta diversity was clearly higher between individual mice compared to the different time points of stool sampling within mice of this study. Principle Coordinate Analysis (PCoA) of weighted and unweighted UniFrac distance matrices did not separate DEREG and wildtype mice gut microbiomes. PCOA clustered samples according to their sample identifier and age rather than according to the different time points before and after Treg depletion.

Conclusion: In this work we analysed the gut microbiome variation associated with Treg depletion in mice over time. Intra-microbiome variability of the gut microbiome over time was less pronounced compared to inter-microbiome variability before Treg depletion.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

445

POSTER PRESENTATIONS

P.D1.04.13
Microbiome composition of air samples from livestock farms and their effect on innate immune receptors and cells

R. Mariman, D. Liu, M. Gerlofs-Nijland, B. John, F. Cassee, E. Pinelli; RIVM, Bilthoven, Netherlands.

Patients with respiratory diseases in rural areas have been reported to have enhanced responsiveness to ambient particulate matter (PM). In addition to the physical and chemical components, ambient PM can contain microorganisms or parts thereof which is referred here as BioPM. The aim of this study is to characterize the microbiomal composition of BioPM originating from livestock, and to investigate whether these BioPM can trigger the activation of innate receptors and cells. Size-resolved BioPM samples (<2.5 and 2.5-10 µm) were collected from chicken, pig and goat farms using the versatile aerosol concentration enrichment system (VACES) connected to a Bioaer sampler. The fungal and bacterial community was assessed with an amplicon based approach using Next Generation Sequencing. In parallel, HEK-Blue cells expressing different pattern recognition receptors (Toll like receptors (TLR) 2, 3, 4, 5, 7, 8, 9 and NOD1,2) and a human monocyte cell line (MM6) were exposed to BioPM from these sites. Results indicate distinct airborne microbiota profiles associated with the corresponding animal farm. Moreover, the various BioPM contained mainly ligands for TLR2 and TLR4 resulting in a concentration-dependent upregulation of inflammatory cytokines secreted by MM6 cells. In addition, the effect of size-resolved PM on the cytokine profile was also investigated. A higher rate of glycolysis and TLR4 only the ligand derived BioPM induced TLR4 activation. These findings indicate that BioPM from livestock can activate innate cells and therefore, trigger inflammatory responses. Knowledge on the microbial composition of BioPM derived from different farms and understanding what type of inflammatory responses they induce is crucial for future studies on the effect of exposure to BioPM may have on respiratory systems.

P.D1.04.14
Lactobacilli secreted factors dampen human IFN-γ responses through a monocyte-dependent mechanism

M. Mata Forsberg, S. Björkander, E. Sverremark-Ekström; The Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm, Sweden.

Lactobacilli are common members of the human microbiome known for their immunomodulatory function. We have previously shown that lactobacilli cell free supernatants (CFS) dampen T cell and NK cell pro-inflammatory cytokine production, proliferation and cytotoxicity, in vitro. However, the mechanisms involved remain elusive. Here, we aimed to investigate how soluble factors present in the lactobacilli-CFS mediate the dampening activity. We stimulated whole human PBMC or PBMC depleted of antigen presenting cells in the presence or absence of size fractionated lactobacilli-CFS. Furthermore, conditioned cell culture media from isolated monocytes pre-treated with lactobacilli-CFS was used in subsequent PBMC stimulations. Cytokine production was analyzed with ELISA and flow cytometry. We show that lactobacilli-CFS contains multiple soluble factors of different molecular size capable of reducing IFN-γ expression in NK cells and several T cell subsets. Moreover, the dampening activity is lost when monocytes, but not B cells, are depleted from the PBMC cultures prior to stimulation. Conditioned cell culture media from isolated monocytes pre-treated with lactobacilli-CFS also resulted in reduced IFN-γ secretion from PBMC. In summary, we show that lactobacilli-CFS dampen pro-inflammatory immune responses via a mechanism that involves soluble factors produced by monocytes.

P.D1.04.15
Metabolic reprogramming towards aerobic glycolysis might control the antigen presentation capacity of CD4+ T cells


Conventional CD4+ T cells have recently been identified as potent inducers of cytotoxic memory CD8+ T cells responses. By a process termed transphagocytosis T CD4+ T cells capture bacteria from infected dendritic cells and degrade it to present bacterial antigens to CD8+ T naive T cells, which proliferate and become cytotoxic in responses. This T CD4+ T cell-mediated antigen presentation generates central memory T CD8+ cells with low PD-1 expression and provide anti tumor protection, highlighting the potential of CD4+ T cells as a tool for cancer immunotherapy. Among the complex field of immunometabolism, the present study aims to determine the association between the metabolic state of transphagocytic CD4+ T cells and their ability to induce bacterial antigens to CD8+ T cells. A higher rate of glycolysis and TLR4 only the ligand derived BioPM induced TLR4 activation. These findings indicate that BioPM from livestock can activate innate cells and therefore, trigger inflammatory responses. Knowledge on the microbial composition of BioPM derived from different farms and understanding what type of inflammatory responses they induce is crucial for future studies on the effect of exposure to BioPM may have on respiratory systems.

P.D1.04.16
X-linked Chronic Granulomatosis: molecular and cellular mechanisms underlying intestinal inflammation

M. Melicicota, M. Chiracca', S. Di Cesare', E. Fontana', S. Guglielmetti, R. Rignoni', F. Rea', V. Marrelli', A. Finocchi, B. Cassani; 1Humanitas Clinical and Research Hospital, ROZZANO (MILANO), Italy, University 'Department of Pediatrics, Children's Hospital Bambino Gesù, Rome, Italy, 1, University 'Department of Pediatrics, Children's Hospital Bambino Gesù, Rome, Italy, 1, University 'Department of Food, Environmental, and Nutritional Sciences (DeFENS), University of Milan, Italy, MILANO, Italy, Italian Istituto di Ricerca Genetica e Biomedica, Milan Unit, CNR, Milan, Italy, 1, University of Milan, Italy, 1, Istituto di Ricerca Genetica e Biomedica, Milan Unit, CNR, Milan, Italy, ROZZANO (MILANO), Italy.

X-linked Chronic Granulomatous Disease is an immunodeficiency disorder of phagocytes, due to defect in the CYBB gene, encoding the gp91phox subunit of the NADPH enzyme, resulting in impaired killing of bacteria and fungi. The enzyme is expressed also in lymphocytes but its functional implication is poorly characterized. Affected individuals develop life-threatening infections with unexplained onset and unexplained autoimmune/autoimmune conditions, involving particularly the intestine. Increased tissue expression of pro-inflammatory cytokines (IL-1β, IL-6, IL-18) beta people the intestinal inflammation in the gp91phox-/- mice. Treg cell frequency in the mesenteric lymph nodes was reduced in mutants as well as the percentage of CD103+ dendritic cells, endowed with tolerogenic functions. Consistently, OVA-specific Treg conversion, analysis in vivo upon adoptive transfer of CD4+ Olii cells, that were antigen-delivered, was affected in gp91phox-/- mice. Interestingly, IgA compartment, crucial to contain gut microbes, was similarly defective in the knock-out mice. PBMC of 13 CGD patients and of 10 age-matched HD were evaluated by flow cytometry. Eight out of 13 patients suffered from IBD with predominant colonic involvement. Analysis at the enrollment showed diminished naive CD8 and CD4 subsets and increased effector memory (CD45RA-CD27- CD45RA-CD27+) cells as well as a slight increase in the NKT subset. Despite normal B cell frequencies, the memory subsets (CD19+CD27+ upregulated and switched memory CD19+CD27+igD+), were all below the normal range values. Studies are underway to investigate the presence of rare genetic variants associated with IBD. Further, gene and protein expression from intestinal biopsies will be correlated with microbiota composition and fecal IgA content.

P.D1.04.17
The influence of sex chromosome-specific gut microbiota on a sexually dimorphic immune response

A. Pettit', J. Franko', E. Ongá', K. Klebent', C. Cuff', R. Schafer'; 1West Virginia University, Morgantown, United States, 1Universidade Federal De Víchosa, Víchosa, Brazil.

Despite being less susceptible to infectious diseases, female are 10X more likely to develop autoimmune diseases than males. Immune-related sex dimorphisms have primarily been attributed to sex hormones, however, XX vs. XY sex chromosome complements and sex-specific gut microbiomes may play a role. To identify mechanisms contributing to distinct male vs. female immune responses the gut microbiome composition of four-core genotypes (4CG) mice and its influence on a sexually dimorphic immune response was evaluated. FCG mice exhibit one of 4 genotypes: XX or XY females (ovaries) and XX or XY males (testes). 4CG mice have been used to identify differences in phenotypes caused by sex chromosome complements, sex hormones, and interactions between the two. 16s rDNA sequencing and metagenomics analysis was performed on DNA isolated from the small intestine of 4CG mice. Distinct populations of bacteria were identified between XX females and XY males, and also between XX females and XY males. Differences in the regulation of gut microbiome composition. Elimination of gut bacteria by antibiotic administration demonstrated a potential role for sex chromosome-specific gut microbiota on the XX-dependent enhancement of heat-killed Streptococcus pneumoniae immune responses after exposure to the bovine, propanol. Future experiments will determine the metabolic by-products produced by the microbiome of 4CG mice and determine if these metabolites influence immune responsiveness in a sex chromosome-dependent manner. Funded by the Department of Microbiology, Immunology, and Cell Biology Research Internship and NIH Grant P20GM103434, WVINBRE.

P.D1.04.18
Psoriasis related changes in skin microbiota composition

Z. Stehlíková', K. Kučerová', T. Štětina', D. Kučerová', J. Hrubčíková', J. Hercogová', L. Jiraskova Zakostelska', B. Doležalová', V. Méduna', M. Kostovčik', P. Gantner', M. Kostovčik', K. Juzlova'; 1Institute of Microbiology of the CAS, v.v.i., Prague, Czech Republic, 1Bambino Gesù Children Hospital, Roma, Italy, 1Institute of Food, Environmental, and Nutritional Sciences (DeFENS), University of Milan, Italy, 1, Istituto di Ricerca Genetica e Biomedica, Milan Unit, CNR, Milan, Italy, 1, University of Milan, Italy, 1, Istituto di Ricerca Genetica e Biomedica, Milan Unit, CNR, Milan, Italy, 1, University of Milan, Italy, 1, Istituto di Ricerca Genetica e Biomedica, Milan Unit, CNR, Milan, Italy, ROZZANO (MILANO), Italy.

Psoriasis is an immune mediated, chronic, inflammatory skin disease of unknown etiology affecting 2-3% of the worldwide population. It is widely accepted that it develops from a combination of genetic and environmental triggors, such as stress, bacterial infection, antibiotics, and diet. However, the role of microbiota in its pathogenesis remains still unclear. The aim of this study was to describe microbiota composition in psoriatic patients and compare it to healthy controls. Methods: We collected samples from the human back skin using two sampling techniques: swabbing, and scraping. Subsequently, we compared microbiota composition of affected psoriatic skin with unaffected contralateral skin from the same patient and with healthy controls after sequencing V1V2 region of 16S RNA on Illumina MiSeq platform. Data were analyzed using QiIME.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.D1.04.19

Human gut microbes are highly susceptible to antimicrobial food additives in vitro

L. Hnricová1, E. Sukova1, T. Hudcovic1, J. Krejsek2, T. Hnrici2;
1Institute of Microbiology, Czech Academy of Sciences, Novy Hradek, Czech Republic, 2Faculty of Medicine in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic.

The aim of this project was to test the hypothesis that antimicrobial food additives may alter the composition of human gut microbiota by selectively suppressing the growth of susceptible gut microbes. To explore the influence of antimicrobial food additives on the composition of the human gut microbiota, we examined the susceptibility of both aerobic and anaerobic gut bacteria to sodium benzoate, sodium nitrite, and potassium sorbate, and their combinations, using a broth microdilution method. The tested bacteria showed a range of susceptibilities to the different food additives, with Bacteroides coprocola and Clostridium tyrobutyricum being particularly sensitive. However, most importantly, we found that gut microbes with known anti-inflammatory properties were mostly susceptible to the antimicrobial food additives, while microbes with known pro-inflammatory or colitogenic properties were mostly resistant. Our data show that some human gut microbes are highly susceptible to antimicrobial food additives. We speculate that permanent exposure of human gut microbiota to even low levels of additives may modify the composition and function of gut microbiota and thus influence the host’s immune system.

Whether the effect of additive-modified gut microbiota on the human immune system could explain, at least in part, the increasing incidence of allergies and autoimmune diseases remains to be shown.

P.D1.04.20

Food preservatives induce Proteobacteria dysbiosis of the human gut microbiota

T. Hnrici2, L. Hnricová1, E. Trckova1, V. Machova1, J. Krejsek1;
1Institute of Microbiology, Czech Academy of Sciences, Novy Hradek, Czech Republic, 2Faculty of Medicine in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic.

The incidence of allergies and autoimmune diseases is increasing worldwide. Recent data suggest that gut microbiota can modulate not only local but also systemic immune responses. In this study, we focus on environmental factors, specifically food preservatives, which may modify the composition of gut microbiota and thus influence host’s immune responses. To address this issue, we have administered either sterile water or water supplemented with additives to wild-type and Nod2-deficient C57BL/6 mice colonized with human microbiota. The daily intake of additives was calculated to match the maximum daily intake reached in human populations in Europe. We have analyzed the effect of additives on microbial composition and diversity by amplification and high-throughput sequencing of the hypervariable regions of the 16S rRNA genes. The resulting sequences were processed using QIIME2 software package. Our results indicate that commonly used food preservatives can decrease the diversity of the human gut microbiota and also trigger Proteobacteria dysbiosis.

P.D1.04.21

European XFEL, The world’s largest and powerful X-ray free-electron laser

D. MEZA, J. Guel, R. Schubert, E. Round, H. Han, J. Makroczcyova, K. Lorenzen, J. Schult;
EUROPEAN XFEL, SCHENEFELD, Germany.

The European XFEL generates ultrashort X-ray flashes 27 000 times per second and with a brilliance that is a billion times higher than that of the best conventional X-ray radiation sources. How it works. To generate the X-ray flashes, bunches of electrons are first accelerated to high energies and then directed through special arrangements of magnets. Electrons are focused down to high energies in a superconducting accelerator. They then fly on a slalom course through a special arrangement of magnets in which they emit laser-like flashes of radiation. Instruments. scientists can make use of sophisticated instruments to carry out their experiments such as SPB/SFX (ultrafast coherent diffraction of single particles: structure determination of single particles like atomic clusters, biomolecules, virus particles, cells and serial femtosecond crystallography, the XFE scientific instrument serve a broad scientific community and embrace several fields of ultrafast X ray science and their applications. Delivering new information serving applications in many fields, the XFEL Biology Infrastructure facility with all its equipment provide versatile tools to significantly increase the feasibility of a wide variety of experiments on biological samples. Scope. The X-ray flashes are so short that scientists are able to use them to film ultrafast phenomena such as the formation or breakup of chemical bonds and also make possible to research the composition and structure of complex biomolecules such as proteins, cells, or membranes on the atomic scale providing insights into their functions, offering important basis for the development of future medicines and therapies.

P.D2.01 Innate Lymphoid Cells

P.D2.01.01

A cell-autonomous role of DOCK8 in development of type3 innate lymphoid cells

R. Aihard1, M. Watanabe1, Y. Fukui1;
1Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan, 2Research Center for Advanced Immunology, Kyushu University, Fukuoka, Japan.

Introduction: RORgt-expressing ILC3s play an important role in the defense against intestinal pathogens and promotion of epithelial homeostasis via production of IL-22. However, the mechanism controlling ILC3 development is not fully understood. In this study, we examined the role of DOCK8, an atypical guanine nucleotide exchange factor for Cdc42, in ILC3 development. Material and methods: Conventional DOCK8 KO mice and control mice were used for FACS analyses and immunohistochemical analyses to examine ILC3 development and intestinal fucosylation. The conditional KO mice lacking DOCK8 in RORgt-positive cell lineage were developed and used to examine its effect on ILC3 development. Results: DOCK8 KO mice exhibited a severe reduction of RORgt+ c-Kit+ Sca1- ILC3s in intestinal lamina propria. Consistent with this, lineage-negative cells producing IL-22 were also reduced. The colitogenic properties of ILC3s were mostly resistant. Our data demonstrate that some human gut microbes are highly susceptible to antimicrobial food additives. We speculate that permanent exposure of human gut microbiota to even low levels of additives may modify the composition and function of gut microbiota and thus influence the host’s immune system. Whether the effect of additive-modified gut microbiota on the human immune system could explain, at least in part, the increasing incidence of allergies and autoimmune diseases remains to be shown.

P.D2.01.02

Enrichment of innate lymphoid cell populations in murine gingival tissue

J. L. Brown1, L. Campbell1, J. Malcolm1, A. Adrasos Planell2, J. Butler1,3, S. Chulshwa1;
1University of West of Scotland, Paisley, United Kingdom, 2University of Glasgow, Glasgow, United Kingdom, 3Glasgow Caledonian University, Glasgow, United Kingdom.

Introduction: Innate lymphoid cells (ILCs) are lymphocytes that act as the first line of immunological defence at mucosal surfaces such as the gut, lungs and the skin. Here, we provide a detailed appraisal of the whole ILC population (group 1, 2 and 3 subsets) in the murine gingivae and the regional lymph nodes (dLNs) draining the oral cavity. Methods: Oral dLNs and gingivae were harvested from mice and processed/digested to obtain single cell suspensions, which were subsequently stained with antibodies for identification of ILCs by flow cytometry. For cytokine profiling of ILCs, cells were stimulated prior to staining. Results: We show that ILCs made up a greater percentage of the whole CD45+ lymphocyte population in the murine gingivae than in the oral dLNs (0.356 ± 0.039% vs. 0.158 ± 0.005%, p=0.001). The gingivae-resident ILCs were more diverse than the oral dLNs, with a significantly greater proportion of CD117+, NKp46+ ILCs (35.19 ± 3.84 compared to 5.03 ± 0.69, p=0.0001). The cytokine profile of ILCs in the gingivae also differed from the oral dLNs; there was a relatively similar proportion of IFN-γ+ and IL-5+ ILCs in the murine gingivae, whereas IL-5+ ILCs predominately populated the oral dLNs. Conclusion: The function of ILCs in the oral cavity is currently unknown; here, we demonstrate that the ILC compartment is enriched, more diverse, and has a different cytokine profile at the gingival surface compared to the oral dLNs. Future work investigating inflammatory oral diseases using mouse models may merit consideration of these ILC populations.
D. Corral, F. Levallois, O. Neyrolles, D. Hudisier; 1Institut de Pharmacologie et de Biologie Structurale, Toulouse, France.

Understanding the immune response to Mycobacterium tuberculosis infection (MtB), the etiological agent of tuberculosis (TB) may help propose innovative therapeutic approaches. In this context, the role of innate lymphoid cells (ILCs), which are a recently identified group of innate lymphocytes, remains unexplored. ILCs are preferentially located at mucosal surfaces where they contribute to controlling immunity. The aim of our project is to analyze the dynamics of ILCs upon MtB infection in the murine model and to understand their contribution to the balance between immune-driven protection and pathology, key parameters in ensuring MtB elimination while preserving functional integrity of the lung tissue during TB. Our data show that, during MtB infection, ILC subsets are differentially recruited to the lungs where they are activated and produce various subset-specific cytokines that may contribute to both pathogen clearance and tissue repair. Moreover, we found that cells of the ILC2 subset progressively display common features with ILC1 during the infection time course, suggesting cell plasticity. We now wish to characterize the mechanisms controlling ILC2 dynamics and plasticity in the lungs, as well as their role in modulating TB pathology and anti-mycobacterial immunity.

P.D2.01.05
Dasatinib skewss in vitro human CD56 bright innate lymphoid cells differentiation towards ILC2
L. Damlé1, 2, E. Montalío1, L. Moretta1, M. Mingari3, C. Vitale1.
1CEBR-(Centre of Excellence for Biomedical Research), Italy, Genova, Italy, 2IRCSS-San Raffaele Scientific Institute, Italy, Milan, Italy, 3ImmunoLabs, Italy, Immunology Area, Pediatric Hospital Bambino Gesù, Rome, Italy.

Our preliminary data suggested that a Rorγt+ and RAG-independent population play an important role in the development of kidney fibrosis. Based on this, we aimed to understand the mechanism by which ILCs sense and respond to crystal deposition and influence the development of kidney fibrosis.

Crystal-induced inflammation is a common manifestation of a variety of genetic and acquired metabolic disturbances. DCs or macrophages sense the crystals, activate the NLRP3-inflammasome and produce cytokines like IL18 and IL1β. These, together with others like IL23, promote local inflammation and induce the development of kidney fibrosis.

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In co-cultures, MSCs stimulated the proliferation and IL-22 production of ILC3s and reciprocally, ILCs enhanced MSC expression of VCAM-1 and ICAM-1. In both directions the ILC3s may contribute to the therapeutic effect of MSCs, we co-cultured human tonsil-derived ILC3s with human bone-marrow derived MSCs in standard or transwell culture plates.

The determinants for MSC responsiveness are unknown. We demonstrated that high frequencies of activated group 3 innate lymphoid cells (ILC3s) before and after aHSCT were ascribed to their ability to suppress the activity of (alloreactive) T cells and to support tissue-repair. However, clinical response rates in patients with GvHD are limited to 50%, and the absolute ILC2 count was significantly higher (P=0.03) individuals with DHF (median 0.6%, IQR 0.33 to 1.03%) were significantly higher (p=0.02) compared to healthy individuals (median 0.36% IQR 0.17 to 0.48%). Further the absolute ILC2 count was significantly higher (P=0.03) individuals with DHF (median 8.52, IQR 5.74 to 14.54 cells/mm³) to compare to DF (median 0.52 , IQR 1.41 to11.14 cells/mm³). However, there was no difference in the proportion of ILC2s in patients with DF compared to those with DHF. Serum IL-13 and IL-5 was not detected in any of the patients, while IL-4 was detected in 7 patients. Conclusion: As the proportion and absolute count of ILC2 in DHF is significantly increased these cells could be playing a role in DENV infections.

In conclusion, we investigated ILC2-deficient mice during onset of infection since ILC2s play crucial roles in mediating early type-2 responses. Noteworthy, ILC2 deficient mice evolve a less prominent detrimental type-2 immunity indicated by reduced amounts of classical markers of type-2 immunity such as IL-4, IL-13 and AAM-markers. Contrary, type-1/3 immunity response is increased in these mice by elevated levels of type-1/3 signature cytokines. Consequently, this phenotypic shift results in more efficient control of infections accompanied by less severe lung pathology and prolonged survival.

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POSTER PRESENTATIONS

P.D2.01.14 First-breath-induced type 2 pathways shape the lung immune environment
S. Soluzzi1,1, A. Gorki2, B. M. Rand3, R. Martini4, S. Scanlon5, P. Stark6, K. Lakovits7, A. Hladi6, A. Koropec8, O. Sharifi9, J. M. Warszawski10, H. Jolin11, J. Mesteni1, A. N. McKenzie1, S. Knapp1,2
1Medical University of Vienna, Vienna, Austria, 2Cemm Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, 3MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, 4Institute of Pathology Uberlingen, Uberlingen, Germany.

From birth onward, the lungs are exposed to the external environment and therefore harbor a complex immunological milieu to protect this organ from damage and infection. We investigated the homeostatic role of the epithelium-derived alarmin interleukin-33 (IL-33) in newborn mice and discovered the immediate upregulation of IL-33 from the first day of life, closely followed by a wave of IL-13 producing type 2 innate lymphoid cells (ILC2s), which coincided with the appearance of alveolar macrophages (AMs) and their early polarization to an IL-13-dependent anti-inflammatory M2 phenotype. ILC2s contributed to lung quiescence in homeostasis by polarizing tissue resident AMs and induced an M2 phenotype in transplanted macrophage progenitors. ILC2s continued to maintain the M2 AM phenotype during adult life at the cost of a delayed response to Streptococcus pneumoniae infection in mice. These data highlight the homeostatic role of ILC2s in setting the activation threshold in the lung and underline their implications in anti-bacterial defenses.

P.D2.01.17 Transcriptional regulation of human innate killer cell and ILC3 development and function by ETS-1
S. Taveirne1, S. Wahlen1, L. Kiekenst2, E. Van Ammel3, K. De Mulder4, H. Roels4, L. Tilleman5, M. Aumerier6, F. Van Nieuwerburgh7, T. Kerrel8, T. Taghorn8, B. Vandekerckhove8, G. Leclercq2
1Hghost University, Ghent, Belgium, 2Universit de Lille, Lille, France.

Introduction: Natural killer (NK) cells and innate lymphoid type 3 cells (ILC3s) are innate lymphoid cells that play a critical role in the immune response against tumor cells and pathogens, and also have important immune regulatory functions. The transcription factor Ets-1 is an important factor in murine NK cell biology as Ets-1-deficient mice show severely reduced NK cell numbers and residual NK cells display decreased functionality. No data regarding the function of ETS-1 in the development of human NK cells is available. In addition, the role of ETS-1 in neither murine nor human ILC3 biology has been investigated.

Methods: In this study, we generated ETS-1-deficient human embryonic stem cell (hESC) clones using the CRISPR/Cas9 technology. In a complementary approach, we generated ETS-1 loss-of-function cord blood hematopoietic stem cells (HSCs) by retroviral transduction of the dominant-negative ETS-1-p27 isoform.

Results: Each ETS-1-deficient human ESC clone displayed defective NK cell differentiation capacity. ETS-1-p27-transduced HSCs had a lower potential to differentiate into NK cells and NKp44+ ILC3, which correlated with increased apoptosis. Residual NK cells showed reduced cytokine secretion and cytotoxic activity. Transcriptome analysis showed that ETS-1 has a dual role in NK cells and NKp44+ ILC3, as it both induces and inhibits expression of NK cell- and ILC3-specific genes, respectively.

Conclusion: Our data show that ETS-1 is a critical regulator of human NK cell and ILC3 development and function and provide important insights in the molecular mechanisms of their biology.

P.D2.01.18 ILC3 NCR++regulate endothelial cell activation through NF-kB
G. Vanoni1, P. Romero, S. Trabaneli, C. Jandus
University of Lausanne, Lausanne, Switzerland.

Innate lymphoid cells (ILCs) represent the most recently identified subset of lymphocytes. Despite their established involvement in inflammatory immune responses, the role of ILC3 remains poorly defined.

Our aim is to assess whether ILC3s might exert an active role in controlling or promoting tumor growth through the interaction with the endothelium. Therefore, short-term in vitro expanded ILC3s isolated from the peripheral blood of healthy donors were used in 3h co-culture experiments with an endothelial cell line (HUVEC, human umbilical vascular endothelial cell line) at 1:1 ratio. The activation state of endothelial cells (ECs) was assessed by flow cytometry, by evaluating the level of surface expression of the adhesion molecules E-Selectin, ICAM-1 and VCAM-1.

Among all ILC subsets, ILC3 NCR++elicited the strongest upregulation of adhesion molecules in ECs, in a contact-dependent manner. By specifically blocking the NF-kB pathway in ECs, the level of expression of adhesion molecules was reverted to basal levels. Pre-exposure of ILC3 NCR++to human bladder carcinoma cells line strongly impaired this capacity. ILC3 NCR++induce the expression of adhesion molecules in ECs via NF-kB pathway. The in vitro ECs-ILCs interaction will be further evaluated to assess its functionality and to identify the molecular players. With the use of tumor-bearing mice, the in vivo relevance of the in vitro findings will be tested to unravel if this capacity of ILC3 NCR++could represent a way for facilitating the immune cell infiltration in the tumor and, therefore, impact tumor progression and/or growth.

P.D2.01.19 Relationship between group 3 innate lymphoid cells (ILC3) and Th17 in human nasopharynx-associated lymphoid tissue and their association with pneumococcal carriage in humans
L. Zaki1, R. Xu1,2, M. S. Ahmed, R. Sharma, S. Leong, N. French, Q. Zhang1
1University of Liverpool, Liverpool, United Kingdom, 2Alder Hey Children’s Hospital, Liverpool, United Kingdom, 3Aintree University Hospital, Liverpool, United Kingdom.

Innate lymphoid cells (ILCs) including ILC3 are increasingly appreciated as being critical in local immune homeostasis and inflammation. Similar to Th17 cells, ILC3 express IL17A and/or IL22. Recent data from animal models suggest there is a reciprocal interaction between ILC3 and T cell responses. It is not known whether any relationship between ILC3 and Th17 cells in human nasopharynx and whether ILC3 contributes to the regulation of pneumococcal carriage in humans. Methods: We have studied the ILC3 and Th17 populations in the nasopharynx-associated lymphoid tissue (NALT) from children and adults following stimulation by PMA or a Staphylococcus extract, and analysed their association with pneumococcal carriage. ILC3 and Th17 frequencies and responses following stimulation were examined by flow-cytometry following staining for lineage markers, CD127, NKp44, c-kit, RORgt, IL17A and IL-22. Results: We showed the ILC3 frequency in NALT was higher in children and mainly express IL22 which was in contrast to the markedly higher Th17 frequency in adults. There was a negative correlation between the IL22-expressing ILC3 and IL17A-producing Th17 cells, and a higher frequency of IL17A-expressing Th17 cells in adults. Further analysis revealed that there was an inverse relationship between the IL22-expressing ILC3 and IL17A-producing Th17 cells, and a higher frequency of IL22-expressing ILC3 and IL17A-producing Th17 cells in adults. Our results suggest there is significant interactions between ILC3 and pathogen-induced Th17 response, and ILC3 may critically regulate Th17 response in human nasopharynx through which mediate bacterial carriage in children.

P.D2.01.20 HIV-1 Infects and depletes innate lymphoid cells via type I interferon pathway
J. Zhao1, L. Cheng1, L. Su1, Z. Zhang1
1Beijing 302 hospital, Beijing, China, 2University of North Carolina, Chapel Hill, United States.

Innate lymphoid cells (ILCs), including ILC1, ILC2 and ILC3 subsets, have been engaged as central players in homeostatic and inflammatory conditions, and correlated with the pathogenesis of multiple human diseases. Our recent studies have found that ILC3s were severely depleted from gut mucosal of patients with chronic HIV-1 infection via Fas/FasL-mediated pathway. Blockade of type I interferon (IFN-I) pathway significantly restored ILC3 loss in humanized mice of type I HIV-infected mice. However, it is not clear whether HIV-1 can infect ILC3s. Here, we found that human ILC3s comprising of CD4+ and CD4- subpopulations were present in various human lymphoid organs but with different transcription programs and functions. CD4+ ILC1s expressed HIV-1 co-receptors and were productively infecting by HIV-1 in vitro and in vivo. HIV-1 infection leads to activation, depletion and functional impairment of ILC3 in humans and in humanized mice model. Highly active antiretroviral therapy (HAART) efficiently rescued the ILC3 numbers and reduced their activation, but failed to restore the homeostatic level (ILC3s) by silencing IFN-α/β signaling also prevented HIV-1 induced depletion or apoptosis in ILC3 cells in vitro and in humanized mice in vivo following HIV-1 infection. Our study identified the CD4+ ILC1 cells as a new target population for HIV-1 infection, and revealed that IFN-I contributes to the depletion of ILC1s during HIV-1 infection.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 449
P.D2.02 NK cells and innate immune mechanisms

P.D2.02.01 CARMIL2 splice site mutation in a patient with warts
F. Ateschke1, R. Jacobs1, G. Ahrenstorff1, A. Dingra1, R. Schmitz1; 2
1Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, Netherlands.
We report a novel homologous splice site mutation in CARMIL2 (RUFPR) gene underlying a primary immunodeficiency with warts. Molecular genetic testing was composed of targeted next-generation sequencing of a panel of IRMut genes. A novel splice site mutation was detected in the CARMIL2 gene. The patient presented with disseminated warts and recurrent infections. Treatment with DAA therapy for HCV resulted in complete viral clearance and resolution of warts. This is the first report of a child with CARMIL2 splice site mutation.

P.D2.02.02 NK cell dysfunctions in chronic HCV infection
R. Jacobs1, J. Huisman1, M. Zandbelt1, D. van der Meijden1, B. Strijbos1, R. van der Kooi1; 2
1Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, Netherlands. 2Department of Virology, Erasmus MC, Rotterdam, Netherlands.
We report two cases of persistent HCV infection in siblings with CARMIL2 gene mutations. In both cases, NKG2C+ NK cells were severely impaired while NKG2D+ NK cells were normal. This is the first report of incomplete NK cell defects in CARMIL2 patients. Results: PCR sequencing of CARMIL2 demonstrated that the mutation in the patient with disseminated warts was CARMIL2 splice site mutation. In the other patient with warts, NKG2C+ NK cells were normal.

P.D2.02.03 Long-term effects of chronic HCV infection on Natural Killer cells
B. W. Babić1, J. Huisman1, M. Zandbelt1, R. van der Kooi1; 2
1Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, Netherlands. 2Department of Virology, Erasmus MC, Rotterdam, Netherlands.
We describe two cases of chronic HCV infection in siblings with CARMIL2 gene mutations. One patient presented with disseminated warts and the other patient presented with isolated HCV infection. We report that NKG2C+ NK cells were severely impaired while NKG2D+ NK cells were normal. This is the first report of incomplete NK cell defects in CARMIL2 patients. Results: PCR sequencing of CARMIL2 demonstrated that the mutation in the patient with disseminated warts was CARMIL2 splice site mutation. In the other patient with warts, NKG2C+ NK cells were normal. We conclude that chronic HCV infection has long-term effects on NK cell function.

P.D2.02.04 Persistent replication of HIV, HCV and HBV results in distinct gene expression profiles by human NK cells
L. Brasseur1, J. Huisman1, M. Zandbelt1, R. van der Kooi1; 2
1Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, Netherlands. 2Department of Virology, Erasmus MC, Rotterdam, Netherlands.
We report two cases of chronic HCV infection in siblings with CARMIL2 gene mutations. One patient presented with disseminated warts and the other patient presented with isolated HCV infection. We report that NKG2C+ NK cells were severely impaired while NKG2D+ NK cells were normal. This is the first report of incomplete NK cell defects in CARMIL2 patients. Results: PCR sequencing of CARMIL2 demonstrated that the mutation in the patient with disseminated warts was CARMIL2 splice site mutation. In the other patient with warts, NKG2C+ NK cells were normal. We conclude that chronic HCV infection has long-term effects on NK cell function.

P.D2.02.05 Mature NKG2C+ NK cells demonstrate increased HLA-DR expression
S. A. Erokhina1, P. A. Kobzeva1, M. A. Streitskova2, E. I. Kovalenko1; 2
1Institute of Bioorganic Chemistry of RAS, Moscow, Russian Federation. 2Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, Netherlands.
We report two cases of chronic HCV infection in siblings with CARMIL2 gene mutations. One patient presented with disseminated warts and the other patient presented with isolated HCV infection. We report that NKG2C+ NK cells were severely impaired while NKG2D+ NK cells were normal. This is the first report of incomplete NK cell defects in CARMIL2 patients. Results: PCR sequencing of CARMIL2 demonstrated that the mutation in the patient with disseminated warts was CARMIL2 splice site mutation. In the other patient with warts, NKG2C+ NK cells were normal. We conclude that chronic HCV infection has long-term effects on NK cell function.

P.D2.02.06 Endometrial natural killer cells reveal a tissue-specific receptor repertoire
D. Feyraets1, T. Kuret1, B. van Cranenbroek1, S. van der Zeeuw-Hingrez1, I. Joosten1, R. G. van der Molen1; 2
1Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, Netherlands. 2Department of Virology, Erasmus MC, Rotterdam, Netherlands.
We report two cases of chronic HCV infection in siblings with CARMIL2 gene mutations. One patient presented with disseminated warts and the other patient presented with isolated HCV infection. We report that NKG2C+ NK cells were severely impaired while NKG2D+ NK cells were normal. This is the first report of incomplete NK cell defects in CARMIL2 patients. Results: PCR sequencing of CARMIL2 demonstrated that the mutation in the patient with disseminated warts was CARMIL2 splice site mutation. In the other patient with warts, NKG2C+ NK cells were normal. We conclude that chronic HCV infection has long-term effects on NK cell function.

P.D2.02.07 Influenza virus-stimulated human NK cells display distinct autocrine immune activation through IL-27 signaling
I. Joosten1, R. Jacobs1, P. A. Kobyzeva1, M. A. Streltsova1, E. I. Kovalenko2; 1ICAR- Central Institute of Freshwater Aquaculture, Bhubaneswar, India. 2Institute of Bioorganic Chemistry of RAS, Moscow, Russian Federation.
We report two cases of chronic HCV infection in siblings with CARMIL2 gene mutations. One patient presented with disseminated warts and the other patient presented with isolated HCV infection. We report that NKG2C+ NK cells were severely impaired while NKG2D+ NK cells were normal. This is the first report of incomplete NK cell defects in CARMIL2 patients. Results: PCR sequencing of CARMIL2 demonstrated that the mutation in the patient with disseminated warts was CARMIL2 splice site mutation. In the other patient with warts, NKG2C+ NK cells were normal. We conclude that chronic HCV infection has long-term effects on NK cell function.

P.D2.02.08 Identification of persistent replication of HIV, HCV and HBV results in distinct gene expression profiles by human NK cells
S. A. Erokhina1, P. A. Kobzeva1, M. A. Streitskova2, E. I. Kovalenko1; 2
1Institute of Bioorganic Chemistry of RAS, Moscow, Russian Federation. 2Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, Netherlands.
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P.D2.02.09 Persistent replication of HIV, HCV and HBV results in distinct gene expression profiles by human NK cells
S. A. Erokhina1, P. A. Kobzeva1, M. A. Streitskova2, E. I. Kovalenko1; 2
1Institute of Bioorganic Chemistry of RAS, Moscow, Russian Federation. 2Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, Netherlands.
We report two cases of chronic HCV infection in siblings with CARMIL2 gene mutations. One patient presented with disseminated warts and the other patient presented with isolated HCV infection. We report that NKG2C+ NK cells were severely impaired while NKG2D+ NK cells were normal. This is the first report of incomplete NK cell defects in CARMIL2 patients. Results: PCR sequencing of CARMIL2 demonstrated that the mutation in the patient with disseminated warts was CARMIL2 splice site mutation. In the other patient with warts, NKG2C+ NK cells were normal. We conclude that chronic HCV infection has long-term effects on NK cell function.
Results showed that the NKR repertoire of eNKs is distinct from pbNKs, with eNKs co-expressing more than 3 NKR simultaneously. In contrast to the pbNKs, expansions of NK subsets in eNKs were independent of GzmB and NKG2D expression. The eNKs were refractory to NKp46-mediated lysis and HLA-C-blocking antibodies, suggesting reduced local turnover of eNKs and/or a distinct licensing process. Taken together, our data reveals that eNKs have a unique tissue-specific signature, suggesting they are finely tuned to accept the semi-allogenic fetus. These findings pave the way for the evaluation of eNK function during pregnancy complications, and may yield insight into their pathogenesis, thereby setting the stage for the discovery of pregnancy-success related biomarkers.

P.D.2.02.07
Early Extracellular Followed by a Late Surge in Intracellular Release of Oxygen Radicals in Rat Neutrophils Following Acute Burn Injury With Sepsis

N. Faizl, M. Ibrahim
Chicago State University, Chicago, United States.

Background: This is a study of neutrophil host defense and neutrophil oxidant production in burn-injured rats with a superimposed E. faecalis infection; it will enhance our understanding of pathogenic mechanisms by which infections exacerbate host defense dysfunction occurring with burn injury alone.

Methods: Our studies focus on alterations in neutrophils’ oxidative nitric oxide in burn rats inoculated with Enterococcus faecalis. Blood and peritoneal PMN were obtained from different experimental and control groups of animals. O2- production both extracellular and intracellular was measured in PMN before and after their stimulation with PMA (100ng/mL) using Isolumin-labeled enhanced luminometry.

Results: We found that there is an early extracellular followed by a late surge in intracellular release of oxygen radicals in rat neutrophils following acute burn injury with sepsis. Blood PMN O2- remained upregulated after infection of rats with E. faecalis superimposed with the burn injury. E. faecalis alone did not cause an increase in blood PMN O2- to the level caused by burn alone. E. faecalis plus burn cause a much greater increase in peritoneal PMN O2- than in blood PMN O2- compared to the increase with burn alone.

Conclusions: These data show tissue levels in the wound caviary as the activation status and the oxidative burst of PMN to be monitored. Our data suggest that the neutrophil is a key player in the consumption of the superoxide radicals of the bloodstream. In addition, we also know that there is a likely exacerbation of extracellular release O2-2 by tissue PMNs in the combined burn and E. faecalis injury. We conclude that a differential kinetics of ROS release in circulatory and tissue neutrophils following burn and sepsis.

P.D.2.02.08
CD127+ In innate Lymphoid Cells (ILCs) with NK cell features accumulate in Inflammatory Bowel Disease (IBD)

L. Krabbenbroeck1, C. Kradolfer1, C. Busken1, W. Bemelman1, J. H. Bernink1, H. Spits1
1Academic Medical Center, Amsterdam, Netherlands; 2Hubrecht Institute, Utrecht, Netherlands.

CD127+ innate Lymphoid Cells are interdependent of prior cytomegalovirus (CMV) infection and HLA-E deficiency. We demonstrate by dysregulated immune responses. In innate lymphoid Cells (ILCs), including helper ILCs and NK cells, are important for intestinal homeostasis and protective immune responses, but can contribute to inflammatory diseases when not properly balanced. Three helper ILC subsets are distinguished by their cytokine profile but each subset adapts its phenotype and cytokine profile in response to environmental cues. This plasticity is extensively described between helper ILC subsets, but the distinction and plasticity between helper ILCs and NK cells requires further investigation.

In human intestinal tissue, we identified an IFN-y-producing cell type that expresses the ILC markers CD127 (IL-7R) and the NK cell marker CD94. Based on phenotype, transcription profile and cytokine secretion this cell type resembles both ILCs and NK cells. In vitro experiments demonstrated that conventional helper ILCs acquire a cytotoxic, IFN-y-producing CD94+ phenotype when exposed to IL-12 that can kill K562 target cells. Since these cytokotic ILCs retain expression of a number of ILC markers and lack some NK markers they remain distinct from NK cells. This indicates that helper ILCs can acquire NK cell features, which may be reversible. Thus, like CD4+ T cells, helper ILCs can acquire features of cytotoxic NK cells and vice versa. These cytokotic CD227+CD16+ ILCs are more prominent in human adult inflamed intestine compared to non-inflamed intestinal specimens and virtually absent in human fetal intestine, suggesting that an inflammatory environment favors accumulation of this cell type. Therefore, these novel cytokotic CD227+CD16+ ILCs may contribute to IBD pathology.

P.D.2.02.09
Murine gamma delta T cells display distinct subpopulations based on their ability to phagocytose unopsonised bacteria

J. C. Lenzo, S. Feng, J. Holden, N. M. O’Brien-Simpson, E. C. Reynolds1
1Oral Health Cooperative Research Centre, Melbourne Dental School, Bio21 Institute, The University of Melbourne, Melbourne, Victoria, Australia, 2Melbourne, Australia.

In humans and mice, gamma-delta (γδ) T cells constitute approximately 1-5% of the total circulating T cells. Despite being a minority in circulation, murine γδ T cells exist as a large proportion in the tissue, including mucosal tissue. γδ T cells can phagocytose unopsonised pathogens, which is critical for immune regulation and infection surveillance are proposed for γδ T cells. Chronic periodontitis, an inflammatory disease of the supportive tissues of the teeth, leads to resorption of alveolar bone and eventual tooth loss. Although chronic periodontitis is associated with a polymicrobial biofilm, specific bacterial species such as Parphyromonas gingivalis are closely associated with clinical measures of disease. Using in vivo mouse models, we show that γδ T cells respond rapidly upon P. gingivalis infection and are able to phagocytose unopsonised P. gingivalis, confirming their role in innate immune responses. We have also identified novel murine γδ T cell subpopulations based on cell surface marker expression and ability to phagocytose bacteria, CD27 γδ T cells are efficient phagocytes while CD227 γδ T cells are less phagocytic and express higher levels of expressed antigen presentation markers. We have also found that human blood γδ T cells can phagocytose unopsonised P. gingivalis, indicating that they may also play a role in the immune response observed in human periodontitis. Based on our findings, we propose that the slower phagocytic CD227 γδ T cells have a more antigen presentation role, whereas the rapid phagocytic CD27 γδ T have a more microbial clearance role.

P.D.2.02.10
Murine γδ T cell functional characterisation of neutrophil granulocytes during Aspergillus fumigatus infection

F. Neumann1, J. Weski1, P. Seddigh2, T. Bracht2, M. Gunzer2
1University Hospital Essen, Institute for Experimental Immunology and Imaging, Essen, Germany; 2Ruhr-Universität Bochum, Medizinisches Proteom-Center, Bochum, Germany.

Invasive Aspergillosis is a common condition in individuals suffering from one of various forms of immune suppression. The cause of this ailment are inhaled conidia of the mould fungus Aspergillus fumigatus, normally cleared from the airways predominantly by neutrophil granulocytes. If the clearing fails, the conidia start to germinate and the hyphal network can spread and invade every major organ. However, the underlying mechanisms making or breaking a successful clearance are still to be identified. By analysing the proteome of neutrophils from mice challenged with conidia, we could identify proteins which are highly regulated in the activated cell, like PAD4 or MPO. The majority of proteins we identified remain as of yet uncharacterised in an infectious context and are now investigated utilizing immobilised Hox88 progenitor cells. We knock out the correlating genes via CRISPR/Cas and the in vitro differentiated Hox88 neutrophils will be examined in an array of methods designed to assess neutrophil functionality, e.g. NET formation or ROS production. We created Hox88 cells which express totoflores only when differentiated into neutrophils. This specific fluorescence permits us to confirm gene function of promising candidates even under physiological conditions by transferring knockout cells into mice and tracking them to verify their role in an in vivo model of pulmonary aspergillosis. In researching the basic mechanisms of the mostly unnoticed conidia clearing process we will be able to bring more understanding to A. fumigatus infection cases and possibly aid in curing, perchance even preventing this life-threatening condition in immunocompromised patients.

P.D.2.02.11
NKGD2 sets activation threshold for NCR1 early in NK cell development and controls sensitivity of cancer immune-surveillance

V. Jelinić1, M. Lenartić1, T. Holmer1, M. Prchul1, V. Sexl1, T. H. Breyesen1, F. M. Wensveen2, B. Polić3
1Faculty of Medicine, University of Rijeka, Rijeka, Croatia; 2Breedegmian Laboratory, Department of Clinical Sciences, University of Bergen, Bergen, Norway; 3Institute of Pharmacology and Toxicology, Department for Biomedical Sciences, University of Veterinary Medicine Vienna, Vienna, Austria, 4Center for Hematology and Regenerative Medicine, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden.

Introduction: NKGD2 and NCR1 (Nkp46) are both activating receptors expressed on all NK cells during NK cell development and have important role in the stress-surveillance. 'Stressed' cells up-regulate NKG2D and/or NCR1 ligands which can engage their receptors and activate NK cells. Previously, our group has shown that NKGD2-deficiency affects NK cell development (Zafirova et al. Immunity 2009). Klrk1-/- and DAP12-/- mice showed an enhanced NK cell-mediated resistance to MCMV infection, while they kept impaired ability to kill NKG2D-expressing tumor targets. Aim: Here we investigated molecular mechanism underlying the NK hyperreactivity and how it influences control of tumors which do not express NKG2D ligands. Materials and Methods: In our research we used two tumor models: 1) radiation-induced thymoma and B16 melanoma. We also used different functional assays, flow cytometry and various genetically modified mice to investigate roles of specific receptors and signaling molecules. Results: NKGD2-deficiency results in specific NCR1-mediated NK cell hyperreactivity. The hyperreactivity occurs during the NK cell development and is due to the lack of signaling through NKG2D-DAP12 axis. It is correlated with reduced expression of CD31 and Zap70. The hyperreactivity results in better control of the investigated tumors and MCMV infection in Klrk1-/- and DAP12-/- mice. Conclusion: This research shows for the first time that an activating NK receptor controls activity of another one. Early during NK cell development NKGD2/DAP12 axis sets threshold for NCR1 which leads to NK cell hyperreactivity and better control of MCMV infection and tumors expressing NCR1 ligands.
Poster P.D2.01.12

Pathway-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells

Q. Hammer1, T. Rücker1, C. Romagnani1,2

1Institute for Immunology, German Rheumatism Research Center (DRFZ), Leibniz Association, Berlin, Germany. 2Charité Universitätmedizin, Berlin, Germany.

Natural killer (NK) cells are innate lymphocytes that lack antigen-specific rearranged receptors, a hallmark of adaptive lymphocytes. In some people infected with human cytomegalovirus (HCMV), an NK cell subset expressing the activating receptor NKp46 undergoes clonal-like expansion that partially resembles anti-viral adaptive responses. However, the viral ligand that drives the activation and differentiation of adaptive NKp46+ NK cells has remained unclear. Here we found that adaptive NKp46+ NK cells differentially recognize HCMV strains encoding variable UL40 peptides that, in combination with pro-inflammatory signals, controlled the population expansion and differentiation of adaptive NKp46+ NK cells. Thus, we propose that polymorphic HCMV peptides contribute to shaping of the heterogeneity of adaptive NKp46+ NK cell populations among HCMV-seropositive people.

Poster P.D2.01.13

Neutrophils suppress mucosal associated invariant T cells

M. Schneider, J. E. Ussher

Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand.

Mucosal associated invariant T (MAIT) cells are abundant innate-like lymphocytes which are rapidly activated in response to bacterial and fungal infections. Upon activation MAIT cells produce cytokines IFNγ and TNFα, and the expression of the MAIT cell surface markers CD161 and CD137 (4-1BB) are crucial for the early immune response. Here we show that polymorphic HCMV peptides, such as y7 T cells and INKT cells. Here, we investigated the influence of neutrophils on MAIT cell activation. MAIT cells, monocytes, and neutrophils were isolated from the blood of healthy donors. Cells were stimulated with fixed bacteria, and their activation assessed by flow cytometry. We show that neutrophils suppressed the activation of MAIT cells, both in cocultures with monocytes and in PBMCs after stimulation with fixed bacteria. The production of effector cytokines INFγ and TNFα, as well as the upregulation of cell surface markers and parasites. In other words, neutrophils suppress the activation of MAIT cells and may play an important role in the regulation of the innate immune response to extracellular bacteria through the inhibition of innate-like lymphocytes. Investigations into the mechanism of suppression are ongoing with results to be presented at the conference.

Poster P.D2.01.14

In vivo depletion of T-bet in intestinal innate lymphoid cells

J. Schroeder1, Jo. L. Roberts2, H. Helmby3, G. Lord4

1End’s College London, London, United Kingdom. 2London School of Hygiene and Tropical Medicine, London, United Kingdom.

Introduction: Innate lymphoid cells (ILC) have been suggested to play important roles at mucosal surfaces primarily by the expression of subset-specific cytokines regulated by lineage-defining transcription factors. However, due to the lack of appropriate mouse models the functional redundancy of ILC in certain models cannot be excluded. Method: We generated T-bet−/− C57/BL6 mice allowing the tamoxifen-induced depletion of T-bet in vivo. Breeding pairs were set up in order to generate Cre-ERT2 positive and negative litters. Tamoxifen was administered via the intraperitoneal route on 5 consecutive days. Mice were rest 3 weeks prior to the injection of tamoxifen. Afterwards cells were isolated from the colon or PRMs for further treatment or reconstituting drinking water or experimental conditions. Results/ conclusions: Here we show that T-bet is crucially important to maintain NKp46+ NK1.1+ CD127− ILC2 in the colon and small intestinal lamina propria. In contrast to CD127+ ILC, T-bet expression in CD4 T cells was only partially diminished. Strikingly, upon tamoxifen-induced depletion of T-bet: CD127+ ILC mice showed significantly less weight loss upon DSS challenge. This observation stands in contrast to models of intestinal infection with N. brasiliensis or H. polygyrus as depletion of T-bet expressing CD127+ ILC did not result in accelerated parasite depletion. Furthermore there was minimal evidence of ILC plasticity following temporally defined T-bet deletion. Hence, our novel model of specific depletion of T-bet in CD127+ ILC points to the crucial role of this transcription factor in mucosal inflammation.

Poster P.D2.01.15

Galectin-3 deficiency promotes liver inflammation and facilitates TNF-α-dependent hepatocyte death in MCMV infection

B. S. Stojanovic1, J. Strazic Geljic1, A. Arsenijevic1, N. Milavenovic1, S. Janjic1, M. L. Lukic1, M. Milavenovic1

1Faculty of medical science, Krusevac, Serbia, 2Faculty of Medicine, Rijeka, Croatia.

Galectin-3 (Gal-3) is a lectin that plays various roles in the pathogenesis of malignant, inflammatory, autoimmune and infectious diseases including liver diseases. In this study, using C57BL/6 mice with target deletion Gal3 (Gal-3−/−) for the first time was demonstrated that the absence of Gal-3 enhanced liver damage in hepatitis induced by intrahepatic murine cytomegalovirus (MCMV). Livers of MCMV infected Gal-3−/− mice contained more inflammatory and necrotic foci, necrotic hepatocytes, and significantly higher level of ALT in the sera compared with the group of C57BL/6 (WT) infected mice, 36 and 72 hours after infection. Significant increase in viral titres was detected in the liver of Gal-3−/− mice compared to WT mice, 72 hours after infection. A significant expression was detected in hepatocytes of MCMV infected mice and it was higher in the livers of Gal-3−/− mice compared with the group of WT mice. The number of TNFα-positive hepatocytes isolated from the livers of infected mice and concentration of TNF-α in liver tissue samples was significantly higher in the group of Gal-3 KO mice. TNF-α blockade with monoclonal antibodies after MCMV infection significantly reduced hepatocyte necrosis only in Gal-3 KO mice. MCMV infection increased the expression of Gal-3 on hepatocytes of WT mice. Treatment with Gal-3 inhibitor (TD139) enhanced liver necrosis in WT mice and administration of recombinant Gal-3 reduced liver inflammation and damage in Gal-3 KO mice. This study demonstrated that Gal-3 plays a role in hepatitis induced with murine cytomegalovirus infection by reducing TNF-α-induced hepatocyte death.

Poster P.D2.01.16

The role of IFNγ in patients with ulcerative colitis

A. Voleviciute1, D. Skotchkodina1, R. Abdulkhakov2, S. Abdulkhakov2, A. Ravanov2

1Vilnius State Medical University, Kaunas, Russian Federation, 2Kazan Federal University, Kazan, Russian Federation.

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) and considered as a chronic disorder of the gastrointestinal tract characterized by intestinal inflammation and epithelial injury. The pathogenesis of UC remains unclear. Disease has been considered to be associated with a non-conventional Th2 response. Besides the role of Th17 in the pathogenesis of autoimmune inflammation in UC is being discussed. Nowadays results from numerous studies indicate a role for innate lymphoid cells (ILC) in the pathogenesis of chronic intestinal inflammation in IBD Objective: To analyze the serum levels of IFNγ in patients both in the acute stage and resolution of UC. Methods: Forty eight patients in the acute stage and twenty patients in remission of UC were included into the study. Serum cytokine levels were analyzed using multiplex immunoassay. Statistical analysis was performed using STATISTICA 6.0 Software Package. Eleven healthy volunteers were included into the control group. Results: Statistically significant increase of IFNγ in patients of both in acute stage (176,15 pg/ml [65,15;359,84]) and remission (42,6 pg/ml [29,4;64,45]) was compared to controls (16,5 pg/ml [12,3;23,2], p=0,0017; 0,0118 respectively). In conclusion: IFNγ might be a marker of functional overactivity of ILC.Conclusions: Increased levels of IFNγ might suggest overactivity of innate lymphoid cells. Innate lymphoid cells may contribute to chronic immune inflammation in the pathogenesis of ulcerative colitis.

Poster P.D2.01.17

Innate lymphoid cells in paediatric inflammatory bowel disease

A. Von Acker1, E. Kvedaraitė1, M. Lourda2, M. Ideström3, J. Henter4, M. Svensson4, J. Måslbärg5

1Center for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden, 2Children’s Cancer Research Unit, Department of Women’s and Children’s Health, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden, 3Pediatrik Gastronomi och Hepatologi, Heparin and Nutrition Unit, Department of Women’s and Children’s Health, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

Background: The underlying etiology of paediatric inflammatory bowel disease (PIBD) remains largely unknown, indicating the dire need for more information on the mechanisms driving this disease. Recent publications have highlighted the importance of ILCs in murine and adult IBD development and progression. In this project, we aim to study ILC heterogeneity and function specifically in PIBD. Methods: Peripheral blood mononuclear cells (PBMCs) and single-cell suspensions were isolated from blood and colon biopsies, respectively, from 29 PIBD and 5 non-PIBD patients admitted to the Pediatrik Gastronomi och Hepatologi and Nutrition Unit at Karolinska University Hospital, Sweden. ILC population frequency and phenotype were examined by 18-colour flow cytometry and correlated to children’s IBD physician global assessment (PGA) scores. Results: Preliminary results from our flow cytometry data show a statistically significant decrease in the frequency of ILC3 cells (p<0.05) and a statistical tendency towards an increase in the frequency of ILC1 (p=0.06) in the gut of PIBD as compared with non-PIBD control patients. In PIBMC, we detected a slight decrease in the frequency of ILC1 (p<0.06). Conclusions: Our data suggest a skew in the balance in the intestinal mucosa of PIBD patients, with an increase of IFNγ-producing ILC1 and a decrease of IL-22-producing ILC3. However, our results are based on a small patient cohort and as yet lack sufficient power for our intended statistical analysis. Extensive further research will now be conducted to continue this analysis as well as examine additional phenotypical and functional differences in ILCs from PIBD patients and non-PIBD controls.
P.D3.01 Novel approaches to vaccinology - Part 1

P.D3.01.01 Investigation of immunogenic properties of Hemolin from Bombbyx mori as carrier protein: an Immunoinformatic approach.
S. Aathmanathan, V. K. Prajapathi, M. Krishnan
1Bharathidasan University, Trichy, India, 2Central University of Rajasthan, Ajmer, India.
Encapsulated bacteria are pathogens which causes disease among elderly, infants and immune-compromised individuals. Since polysaccharides (haptens) are less immunogenic, conjugated vaccines are conjugated with haptens to elicit a stronger immune response and prolonged T-cell memory. dendritic cells are the most potent antigen presenting cells (APCs). Toll like receptors (TLRs) present on APCs plays a major role in identification of antigen and activation of cell mediated immunity. Hence TLR agonists are potential immunostimulants. Carrier protein which enhances the cell mediated immunity through TLR activation is a potent candidate for conjugate vaccine. Hemolin, 48 kDa protein which is present in major lepidopteran insects, that is similar to mammalian immunoglobulin. It has also a natural affinity towards the bacterial lipopolysaccharides. In the present study, Hemolin was modelled using RaptorX. The model was validated using RAMPAGE and ProsaWeb servers. LPS of Er. coli, TLR3 and TLR4 of Homo sapiens were docked with Hemolin using Patchdock and interaction was visualized using Chimera 1.11. Docked complexes were subjected to molecular dynamics for 20ns with GROMACS standalone tool. B and T-cell epitopes were identified using IEDB server. The allergenicity was predicted using AllerTop and AllerFP servers. This makes hemolin a suitable candidate as a carrier protein in conjugate vaccine because of its high immunogenicity and TLR activation.

P.D3.01.02 In silico designing of a novel multi-epitope peptide vaccine against Leishmania infantum: Analysis of its immunogenic potential in vitro and in vivo
M. Aguiló, E. Athanasiou, M. Manganari, E. Karagouni
Laboratory of Immunobiology and Microbiology, Hellenic Pasteur Institute, Athens, Greece.
In the present study, we have designed a multi-epitope peptide vaccine referred as LiChimera, containing several Helper (HTL) and Cytotoxic (CTL) T lymphocyte epitopes obtained from different Leishmania infantum proteins through computational vaccinology approaches. The selected epitopes were fused together by using appropriate linkers, while the N-terminal domain of Heparin-Binding Hemagglutinin (HBHA) from Mycobacterium tuberculosis was also linked as a TLR4 agonist. LiChimera was effectively expressed in E. coli system and its immunogenicity was determined by injecting it intramusically alone or in the presence of Addavax - a squalene-based adjuvant - twice at 2 weeks intervals in BALB/c mice. Results indicated that LiChimera was highly immunogenic and elicited antigen-specific adaptive immune responses, as shown by the high level of serum IgG production and splenocyte proliferation, which were further enhanced in the presence of Addavax. In addition, bone marrow derived macrophages obtained from mice that received LiChimera and Addavax demonstrated increased leishmanial activity confirmed by reduced parasite load in comparison to macrophages obtained from control mice. Overall, we described a new multi-epitope peptide vaccine and its immunogenic properties as a candidate vaccine against leishmaniasis. Further experimentation will be conducted in order to determine its protective efficacy against infectious challenge of Leishmania. This research was made possible through the support of the Savvas Narchos Foundation to the Hellenic Pasteur Institute, as part of the Foundation’s initiative to support the Greek research center ecosystem.

P.D3.01.03 Adjuvants enhance induction of germinal center and antibody secreting cells spleen of neonatal mouse and their persistence in bone marrow
A. A. Aradottir Pind,2 M. Dubik,3 S. S. Thorsdottir,4 J. Holmgren,5 A. Meinke,6,7 G. Del Giudice,8 S. F. Bjarnarson,2 J. Ionsdottir,2,9
1Department of Immunology, Landspitali, the National University Hospital of Iceland, Reykjavik, Iceland, 2School of Health Sciences, University of Iceland, Reykjavik, Iceland, 3Karolinska Institute, Stockholm, Sweden, 4University of Gothenburg Vaccine Research Institute (GUVAX), Department of Microbiology and Immunology, University of Gothenburg, Gothenburg, Sweden, 5Volneva Austria GmbH, Vienna, Austria, 6GSK Vaccines, Siena, Italy.
Introduction: Immunity of the immune system contributes to poor vaccine responses in early life. Germinal center (GC) activation is limited due to poorly developed follicular dendritic cells (FDC), causing generation of few antibody-secreting cells (ASCs) with limited survival and transient antibody responses. The potential of five adjuvants to overcome limitations of the neonatal immune system to induce more robust and prolonged vaccine responses was explored. Materials and methods: Neonatal mice were immunized with a pneumococcal conjugate vaccine Pnc1-TT w/wo adjuvants LT-K63, mmCT, MF59, IC31 or alum. Spleen, bone marrow (BM) and blood were collected at various time points after immunization. Spleen sections were stained for FDC maturation and GC activation, vaccine-specific AbSCs were enumerated in spleen and BM with ELISPOT and vaccine-specific serum-antibodies measured with ELISA. Results: In mice with Pnc1-TT with IL-63, mmCT, MF59 or IC31 we have significantly enhanced maturation of FDCs compared to mice immunized with vaccine alone. LT-K63, MF59 and IC31 significantly enhanced GC formation and mmCT and MF59 significantly enhanced vaccine-specific ASCs in spleen 14 days after immunization. Neonatal mice immunized with Pnc1-TT with LT-K63, mmCT, MF59 or IC31 had significantly enhanced numbers of vaccine-specific ASCs in BM 9 weeks after immunization and significantly enhanced vaccine-specific serum antibodies persisting above protective levels against pneumococcal bacteremia and pneumonia. Conclusion: LT-K63, mmCT, MF59 and IC31 overcame limitations of the neonatal immune system and enhanced both induction and persistence of protective immune response when administered with Pnc1-TT. They are therefore promising candidates for further research on neonatal immune responses.

P.D3.01.04 Immunogenicity of DNA vaccines delivered by patches &lt electroporation in pig skin
1UMR-INRA-Université Paris-Saclay, Jouy-en-Josas, France, 2GABI-INRA-AgroParisTech-Université Paris-Saclay, Jouy-en-Josas, France, 3School of Pharmacy, University College Cork, Cork, Ireland, 4Vaccine Formulation Laboratory, Department of Biochemistry, University of Lausanne, Epalinges, Switzerland, 5INRA, U1177, Plate-Forme d’Infectiologie Expérimentale, PEFIE, Nouilly, France, 6CEA-Université Paris Sud-INSERM, U1184 « Immunology of viral infections and auto immune diseases », IDMIT department, IBI, Fontenay-aux-Roses and Kremlin-Bicêtre, France, 7School of Pharmacy, University College Cork, Cork, Ireland.
DNA vaccines show suboptimal efficacy in humans and domestic species. The delivery of DNA vaccine is key to improve immunogenicity. As skin is a readily accessible tissue rich in antigen presenting cells and pig skin is a relevant model for humans, we explored different modes of DNA delivery in pig skin, i.e. surface electroporation (EP) and dissolvable microneedle patches (DMN), and we assessed the benefit of DNA adsorption on cationic poly(lactic-co-glycidic) acid nanoparticles (NPs). We used plasmids encoding for luciferase and vaccine plasmid encoding for weak antigens derived from the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), an arterivirus responsible for reproductive disorders in sows and respiratory illnesses in pigs. All methods were successful at inducing luciferase expression in skin. At 24 hours post administration, EP, and not DMN, induced a strong recruitment of granulocytes in skin, with the reduction of conventional dendritic cell subsets (cDC1 and cDC2) and of Langerhans cell, in association to a high production of IL1b and IL8. Substantial T cell responses against the PRRSV antigens were induced upon delivery with EP and DMN. Notably, we observed the broadest INFγ T response against a large panel of PRRSV antigenic regions with DNA adsorbed on NP and delivered by EP. Good systemic and mucosal IgG responses were induced by EP and not by DMN delivery. Altogether, delivery of DNA vaccines with electroporation and patches can be achieved in pig skin with variable degrees of transduction efficiency and local inflammation and can induce antigens responses against weak antigens.

P.D3.01.05 Bipolymer based Novel Nanoparticles in Microsphere System as Vaccine Adjuvant
S. Bhargava, V. Bhargava
1Himalayan University, Kanpur, India, 2GTB Hospital, Kanpur, India.
Nanoparticle vaccination is a major strategy for the achievement of safe and effective immunization beyond conventional strategies. Frequent booster dosing can be avoided by development of mucosal/adjuvant vaccine delivery system, which can produce both humoral and cell-mediated responses. The work envisaged uses combined hydrophilic/gelatin nanoparticles, GSN with a hydrophobic polymeric system (PLGA microspheres) which creates a biodegradable system for HBsAg delivery. GN & PLGA microspheres were prepared by double emulsification method and composite system by phase separation method. Composites were optimized and characterized in vitro for their shape, size by Scanning & Transmission Electron Microscopy, Nantagnet entrapment and stability. Fluorescence microscopy was carried out to confirm the uptake. In-vivo study comprised of estimation of IgG response in serum and siG in various body secretions using specific ELISA. The in-vivo studies exhibited an initial burst release from gelatin nanoparticles, degradation of antigen from PLGA microparticles and a continuous release from composite system. This supports the hypothesis & a continuous drug-release from composite system. The fluorescence studies showed the selective uptake of composites by NALT. Humoral response generated by single dose of composites was comparative to marketed formulation receiving booster dose. Further, composite system generated effective siG antibody which was not elicited by marketed formulation. Thus, it could be concluded from present study that bipolymer based composite system are capable to provide sufficient protein stability and can be a promising candidate for development of single shot vaccine, not only against Hepatitis but against all those diseases that invoke host by mucosal surfaces.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.D3.01.06
Oral combination vaccine against Anthrax & Hepatitis B: Development & Characterization
M. Bhargava, S. Bhargava;
1GTB Hospital, Kanpur, India, 2Himalayan University, Kanpur, India.

Infections are still leading cause of morbidity and mortality and most of which can be prevented by vaccination. However, there are too many vaccines to be administered, increasing cost of immunization. Combination vaccines can answer these problems by development of single vaccine containing all possible antigens. The goal of present study was to see the effect of 2 antigens when given in combination. Biolosomes can provide needle free, painless approach for immunization. Recombinant hepatitis B surface antigen(HBsAg) and recombinant protective antigen(rPA) were candidate antigens.

Biolosomes containing rPA and HBsAg were prepared by lipid cast film method. Antigen loaded bilosomes were characterized in vitro for shape, size, antigen entrainment and stability in various body fluids. Fluorescence microscopy was done to confirm the uptake of bilosomes. The in-vivo study comprised of immunization of Balb/c mice and estimation of IgG response in serum and splg in various body secretions using specific ELISA.

Bilosomes formed were multilamellar and stable in gastric and intestinal fluids. Fluorescence microscopy suggested that bilosomes were taken up by gut associated lymphoid tissues. In-vivodata demonstrates that combination produced both systemic as well as mucosal antibody responses upon oral administration at higher dose levels as compared to intramuscular immunization but fail to produce any synergetic effect. When rPA and HBsAg given in combination, HBsAg(high dose) potentiates the production of anti-rPA antibody. Also they elicited measurable splg in mucosal secretions, while alum adjuvanted sugars failed to elicit such responses. The combination produced both systemic as well as mucosal antibody responses upon oral administration.

P.D3.01.07
Myxococcus indicus pranii (Mw) in combination with heat induced promastigotes persuade host protection against drug- sensitive and - resistant Leishmania donovani infection: Activation of IFNα preDC leading to IL-6 producing CD11c+ cDC
S. Dey, D. Mukherjee1, S. S. Sultana, S. Mollick2, S. Mandal2, A. Dutta1, P. Patra2, B. Saha1, C. Pal2;
1West Bengal State University (work station address) Barasat Subdivisional Hospital, Kolkata, India, 2West Bengal State University Dept of Zoology, Kolkata, India.

Introduction: The major concerns of currently available chemotherapy regimens against VL are severe toxicity & resistance. The aim of this work is to design a successful adjuvant in combination with Mw & HIp against experimental murine VL.

Methods: 5 Balb/c mice, per group, were administered with s.c. injection of Mw (10⁶ cells/ kg b.w) & HIP (50 μg / kg b.w) for 7 days after establishing infection. Organs were harvested, assessed by flow cytometry & RTPCR analysis.

Results: Mw+HIP effectively reduced the hepatic & splenic parasite burden of infected animals by inducing CD4+FN+γ T cells, along with the upregulation of Th1 promoting cytokines, chemokines & IL12. We demonstrated that this therapy requires the cooperation of an integrated TLR4/Cytokine/Checmokine loop. We established the roles of IL-6 & IL-12p40, as critical cytokines that mediate anti-id host protection. Mw+HIP induced the expansions of CD11c+CD11b+, CD11c+CD8α+ splenic cDCs & CD11c+CD11b++CD120a+CD205+BDCA1+ splenic pDCs along with the up regulated expressions of IL-6 & IL-12p40. Mw+HIP restored the depleted bone marrow system, in vivo & could direct bone marrow CD11c preDCs, ultimately repopulating the DCs. It also increased cDC1 expression and produced host protective responses by diminishing the CD4+ CD25+ Foxp3+ IL-10+ TGF-β regulatory T cells, and increasing the CD4+ IL17+ Th17 cells in vivo. Interestingly, Mw+HIP were found effective against Miltefosine resistant-L. donovani (HePC-R) in vitro & in vivo as evidenced by the concomitant surge in INOS level & limited expression of amastigote specific Ld-KDNA.

Conclusion: This novel combinational therapeutic cures murine VL by the pro-inflammatory host protective immune responses.

P.D3.01.08
How to improve diphtheria vaccination for the elderly
M. Grasse, A. Meryk, C. Miglitsch, B. Grubeck-Loebenstein;
Institute for Biomedical aging research, University of Innsbruck, Innsbruck, Austria.

We previously demonstrated booster vaccinations with multivalent tetanus/diphtheria vaccines provided long-term protection to tetanus, while long-lasting immunity against diphtheria was insufficient in humans, particularly in the elderly. To investigate the reason for that, we set up a mouse model with different vaccination regimens, consisting of varying numbers of primary and booster vaccinations. Furthermore, we targeted dendritic cells(DCs) by application of GMCSF, and measured humoral & cellular immune responses by ELISA and flow cytometry. For tetanus we can show, that animals who received a primary immunization with three shots of infantfix® and additionally three booster shots with Boosterix® had the same antibody titers than animals which received only three booster shots with Boosterix®. The diphtheria-specific antibodies were much lower of the mice that received only booster shots, compared to the mice with primary and booster shots. By applying GM-CSF next to the vaccine, young and old mice had significantly better diphtheria-specific antibody responses. GM-CSF treated mice had more diphtheria-specific CD4+ T-cells producing IL-2, IL-6 and TNF-α. GM-CSF was leading to a higher number of DCs at the injection-site 24h after vaccination and also to more splicen DCs with upregulated MHC-II expression. Our findings demonstrate, that the imbalanced level of protection against tetanus and diphtheria provided by multivalent tetanus/diphtheria vaccines is most likely due to the vaccine composition and not because of the vaccination regimes. Moreover, targeting DCs with GM-CSF improves the diphtheria-specific immune response and this might be a useful strategy to improve the vaccination situation for the elderly.

P.D3.01.09
Novel RNA adjuvant transcribed from viral internal ribosome entry site improves vaccine efficacy enabling immune-stimulatory capacities
Catholic University of Korea, Seoul, Korea, Republic of.

Most of the vaccines use aluminum compounds (alum) as an adjuvant to increase adaptive immune response to antigens. However, alum-based adjuvants strongly induce Th2 type immune responses rather than Th1 response. Here, we developed a novel single-stranded RNA adjuvant to induce balanced Th1 and Th2 responses. The plasmid containing the gene of interest under the internal ribosome entry site of the cricket paralysis virus intergenic region was transcribed by T7 RNA polymerase. The RNA adjuvant treated bone marrow derived dendritic cells(DCs) T-cell co-culture. Finally, the impact of 2'FL on the gut microbiota composition was evaluated.

Discussion: In vivo study comprised of immunization of Balb/c mice and estimation of protection against experimental murine VL by the pro-inflammatory host protective immune responses.

P.D3.01.10
Human milk oligosaccharide improves innate and adaptive immunity and alters gut microbiota composition in an influenza-specific murine vaccination model
X. Ling;
Utrecht Institute for Pharmaceutical Sciences, Utrecht, Netherlands.

Human milk is uniquely suited to provide optimal nutrition and immune protection to infants. 2'-fucoylsaccaride (2'SF) is one of the most predominant oligosaccharides present in human milk and associated with the immune benefits of human milk. The effect of 2'SF on vaccine responsiveness and mechanisms involved was determined.

A dose range of 0.25-5% (w/v) dietary 2'SF was provided to a murine influenza vaccination model. Vaccine-specific delayed-type hypersensitivity (DTH), antigen-specific antibody levels in serum, and immune cell populations within several organs were evaluated. The effects of 2'SF on vaccine-specific T cell proliferation and cytokine secretions, and the direct immunomodulatory effects of 2'SF were assessed using ex vivo bone marrow-derived dendritic cells (BMDCs) T cell co-culture. Finally, the impact of 2'SF on the gut microbiota composition was evaluated.

Diety 2'SF effectively enhanced vaccine specific DTH responses and serum vaccine-specific IgG1 and IgG2a levels in a dose-dependent manner. Consistently, higher frequency of B2 cells was detected in mice receiving 2'SF. Moreover, proliferation of vaccine-specific CD4+ and CD8+ T-cells, and IFN-γ production after ex vivo restimulation were significantly increased in spleen cells of mice receiving 2'SF, which were in line with changes detected within DC populations. Direct effect of 2'SF on the maturation status and antigen presenting capacity of BMDCs was confirmed in vitro. And the microbiota profile was significantly changed by 2'SF.

Dietary intervention with 2'SF improves both humoral and cellular immune responses to vaccination in mice, which might be attributed both direct immunomodulatory effects and microbiota modification of 2'SF.
POSTER PRESENTATIONS

P.D3.01.11
Immunostimulatory monocytes differentiate into mature antigen presenting cells ex vivo by oligomannose-coated liposomes
Y. Matsuzaki, Y. Kawachi, K. Kuroda, K. Kawachi, A. Tokiyama, N. Kojima;
1Tokai university, Hirusaka, Japan, 2Tokyo Women’s Medical University, Tokyo, Japan.

Oligomannose-coated liposomes (OMLs), in which the antigens are entrapped, have been shown to serve as effective antigen delivery vehicles and as a novel adjuvant to induce antigen-specific cellular immune response. Here, we demonstrate that immunostimulatory monocytes in PBMC can differentiate into mature professional antigen presenting cells ex vivo in response to uptake of OMLs.

When PBMC from C57BL/6 were co-cultured with OMLs in the presence mouse serum, OMLs rapidly was incorporated to CD11b+/Ly6C- mouse inflammatory monocyte. In addition CD86, CCR7, CD83, and MHC class II significantly was upregulated within 24 h after OML uptake. OVA-encoding OML-ingesting monocytes can activate CD8+ T cells from OT-1 mice, suggesting that antigens encapsulated in OMLs were cross-presented in immunostimulatory monocytes. Adoptive transfer of the monocytes that engulf OVA-encoding OMLs led to induction of an antigen-specific Th1 immune response in mice. We also observed that OMLs were preferentially incorporated into human CD14+ monocyte in vitro. In response to in vitro OML uptake, CD14+ monocytes matured accompanied with enhanced expression of HLA-DR and CD86, and with secretion of IL-12. Furthermore, CD209, CD123, and CD169 were expressed on CD14+ monocytes in response to OML treatment. Taken together, mature antigen presenting cells, which can activate CD8 T cells, is generated from immunostimulatory monocytes in peripheral blood by ex vivo treatment of the cells with OMLs without any additional stimuli.

P.D3.01.13
Characterization of a nucleoside-modified mRNA vaccine against HIV-1
1University of Pennsylvania, Department of Medicine, Philadelphia, United States, 2Duke University Medical Center, Department of Surgery, Durham, United States, 3Washington National Primate Research Center, University of Washington, Seattle, United States, 4Department of Pharmaceutics, University of Washington, Seattle, United States.

Introduction: Great progress has been made in understanding the mechanisms of HIV-1 infection, but no effective vaccine worthy of clinical development has been developed to date. In recent years, numerous studies have demonstrated that mRNA-based vaccines could elicit potent immune responses against various infectious pathogens. We have generated a vaccine platform where nucleoside-modified RNAs were encapsulated into lipid nanoparticles (LNPs), which have recently proved to be safe and efficient tools for in vivo nucleic acid delivery. Materials and Methods: Rhesus macaques were intradermally immunized five times with clade C HIV-1 1086 envelope (Env) gp160-encoding nucleoside-modified RNA, in lipid nanoparticles (LNPs) and virus neutralization assays (standardized TZM-bi luciferase reporter system).

Results: Animals generated anti-gp120-specific antibodies, as measured by ELISA. Potent neutralization activity against a highly neutralization sensitive clade C Tier 1 virus (MW965.26) was detected in all animals after four immunizations. Importantly, 3 out of 6 animals developed neutralizing antibodies against the autologous Tier-2 virus (Ce0186, Bl42). Notably, one animal generated antibodies with low neutralizing activity against heterologous clade C Tier-2 strains (28710-2.43 and Ce1176_A3).

Conclusions: Our results demonstrate that antigen-encoding nucleoside-modified mRNA complexed in LNPs induces Tier-2 neutralizing antibody responses in non-human primates. Further characterization of anti-HIV immune responses in this study is underway.

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P.D3.01.15
Zika virus DNA vaccine construction and evaluation of its immunogenicity in mice
E. Prompetchara, P. Bamrungchoaaksom, B. Sukwatsil, K. Kettoy, K. Ruxunyothin;
1Department of Biochemistry and Microbiology and Vaccines and Therapeutic Research Group (STAR), Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, 2Chuloo Vaccine Research Center (ChuloVRC), Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Major ZIKV outbreak occurred during 2015-2016 worldwide. Although most of the ZIKV infections are asymptomatic, unfortunately, there are several reports on severe complications such as microcephaly in newborns and Guillain Barre Syndrome in adults. Currently, either specific treatment or approved ZIKV preventive vaccine are not available. This research, therefore, emphasizes on the study and development of the DNA vaccine against ZIKV as it is easy and fast to produce, thermostable and efficient in immune induction especially when administered with the effective delivery systems. ZIKV pre-membrane and envelope protein was analyzed from ZIKV isolates deposited in the GenBank database. The humanized codons of the consensus ZIKV preM/EN were subcloned into the mammalian expression vector (pCMVkan). The protein expression in mammalian (Vero) cell transformed with pCMVkan encoding ZIKV preM/E was investigated. ZIKV envelope protein was detected extra- and intracellularly when analyzed by western blot and indirect immunofluorescence, respectively. The vaccine was then immunized in Balb/c mouse model using intradermal route in both back and tail. The T cells responses were detected since week 4 (Geometric mean titer, GMT = 10). NAb levels gradually increased at week 6 and week 8 (GMT = 40 and 80, respectively) and are significantly different from those in control group (p<0.05). Collectively, this vaccine prototype is immunogenic in mice and warrant further studies for as an effective ZIKV vaccine.

P.D3.01.16
Humoral and cellular immune response induced by novel liposomal formulations using aminoacid amphiphiles and CpG-ODN as immunostimulants
1Unidad de Investigación Experimental, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina, 2Consejo Nacional de Investigaciones Científicas y Técnicas, Federal Capital, Argentina, 3Laboratorio Inmunología, Tissus Epithéliaux et Cytokines (LITIEC), Université de Poitiers, Poitiers, France, 4Laboratorio de Química Aplicada (LAQ/UIAPA), Dto. Química Orgánica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina, 5Laboratorio Inmunología, Tissus Epithéliaux et Cytokines (LITIEC), EA 4331, Université de Poitiers, Poitiers, France, 6CHU de Poitiers, Poitiers, France.

Introduction: Liposomes are vaccine adjuvant systems able to transport hydro- and liposoluble molecules, allowing the co-incorporation of antigen and different immunostimulants. Our aim was to evaluate the administrability of a cationic liposomal formulation (Lip) with the addition of a CpG oligodeoxynucleotide (CpG-ODN) and/or an aminoacidic amphiphile (Gem) as immunostimulants, in the immunization against recombinant clumping factor of Staphylococcus aureus (rClf).

Materials and Methods: Balb/c mice were immunized with: Lip+CpG-ODN+CIF, Lip+Gem+CIF, or Lip+Gem+CpG-ODN+CIF. Mice received three intraderal doses and were sacrificed three days after the last injection. Anti-CIF IgG was evaluated by indirect ELISA in plasma. Lymph nodes cells were analysed by flow cytometry to assess IL-4, IL-17 and IFNγ production in CD4+ and CD8+ T cells.

Results: Lip+CpG-ODN+CIF, Lip+Gem+CIF and Lip+Gem+CpG-ODN+CIF all led to high IgG levels. Formulations containing Gem or CpG-ODN increased the number of CD4+ lymphocytes. Lip+Gem+CpG-ODN+CIF significantly enhanced CD4+ cells compared to Lip+CpG-ODN+CIF. A similar trend was found for CD8+ lymphocytes. Gem and CpG-ODN were able to induce the production of IL-4 and IFNγ by CD4+ cells and IFNγ by CD8+ cells. Only Gem increased the production of IL-17 by CD4+ cells. Lip+Gem+CpG-ODN+CIF induced the highest number of CD4+ and CD8+ cells producing the three cytokines compared to Lip+CpG-ODN+CIF.

Conclusions: Both Gem and CpG-ODN act as immunostimulants when introduced in liposomes, but their combination enhances the stimulant effect. It should be noted the cytotoxic cells stimulation by the Lip+Gem+CpG-ODN+CIF formulation, considering the impact of this profile in the design of viral or cancer preventive vaccines.

P.D3.01.17
Immunogenetic properties of single component adjuvant formulations
L. Rassmann, A. Ulich, K. Lindt, S. Shorte, M. Hasari, B. David-Watine, M. Bastian, G. van Zandenbergen;
1Paul Ehrlich-Institut, Langen, Germany, 2Institut Pasteur, Paris, France, 3Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany, 4University Medical Center, Maastr, Germany.

An appropriate adjuvant is of pivotal importance for vaccine efficacy and thus should be selected on the basis of the adjuvant’s immunogenetic modes of action. We hypothesize that adjuvant properties can be correlated to specific innate cellular responses influencing the resulting adaptive immune response. Here, we aim to investigate the immunogenetic properties of different single component adjuvants in vitro, comprising TLR agonists, aluminium salts, oil-in-water emulsions and saponins. We focus on side-by-side comparison of the adjuvants using human primary immune cells of 30 donors which are evenly distributed in sex and age.

In a first step, all adjuvants were tested for their pyrogenicity using an in vitro monocyte activation test. Except for MPLA, all tested adjuvants were non-pyrogenic. The capacity of the adjuvants to induce lymphocyte proliferation was examined in a co-culture based assay composed to induce of human monocyte-derived dendritic cells and autologous peripheral blood lymphocytes (PBLs). We observed that Pam3CSK4, Gardiquimod, R848, synthetic MPLA, natural-derived MPLA and TDA induced antigen-independent proliferation of PBLs to varying degrees, with R848 being the strongest stimulator. More detailed examination of B, NK, NKT, CD4+ and CD8+ T cells within the proliferated PBL population revealed that each adjuvant promoted the proliferation of different cell types. An ongoing Luminesx multiplex analysis will uncover the cytokine profile within the co-culture in the first 24h of adjuvant stimulation.

In this study, we will work to identify immunogenetic properties of adjuvants and will facilitate the rational design of adjuvant-based vaccines.
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The complementary determining region three (CDR3) of the light chain of these mAbs were longer than average in rabbits, resembling characteristics of previously isolated DNAVs. Neutralizing rat-elicited and ELISA revealed an epitope at the bottom of the Env trimer targeted by the majority of the isolated mAbs, indicating the immunodominant site of the AMCV008 Env trimer. However, the observed approach angle of these mAbs would lead to a clash of the Fc portion with the viral membrane. Nevertheless, homologous AMCV008 neutralization, and neutralization of another subtype B virus was observed for four antibodies, potentially achieved by destabilization of the Env trimer on the viral surface. In conclusion, mAbs elicited by AMCV008 Env trimer immunizations target the immunodominant bottom of the Env trimer. Viral variability and the suboptimal angle of approach provide an insight in the poor neutralization breadth and should guide future vaccine design.

P.D.3.02 Novel approaches to vaccination - Part 2

P.D.3.02.01
Riboflavin supplementation enhances antigen specific humoral and cellular immune responses to oral vaccines
A. S. Alburti1*, C. McIntee2, S. Longet1, A. Svennerholm1, J. Holmgren2, E. Lavelle1;
1University of Dublin, Trinity college, Dublin, Ireland; 2Qassim University, Al Qassim, Saudi Arabia; 3University of Manchester, Manchester, United Kingdom; 4University of Gothenburg, Gothenburg, Sweden.

In contrast to injectable vaccines, effective mucosal vaccines can provide protective local and systemic immunity. Oral vaccination in particular has the potential to offer a safer and more efficacious approach than injection-based approaches, especially in developing countries. Nevertheless, in general, antigen delivery via the oral route triggers weak immune responses or immunological tolerance. The effectiveness of oral vaccination can be improved by co-administering adjuvants. However, a major challenge is the absence of potent and safe oral adjuvants for clinical application. A second obstacle is that responses to oral vaccines vary between developed Western countries and developing countries where they are often less effective. This may be attributed in part to nutritional deficiencies including a limited intake of factors including vitamins, impacting on the microbiome and the generation of specific metabolites. Here, the immunomodulatory potential of oral supplementation with riboflavin (Vitamin B2) was investigated. It was found that oral delivery of the vitamin enhanced antigen-specific Th1 and Th17 responses in draining lymph nodes in addition to antigen specific antibody responses in mice orally vaccinated with the heat killed oral vaccines against enteric bacterial infections. Prior to and during vaccination with oral cholera vaccine enhanced antigen-specific immune responses in the serum humoral immunity. These findings suggest the potential of riboflavin supplementation to enhance the capacity of oral vaccines to trigger mucosal and systemic immunity.

P.D.3.02.02
Sensitivity of the bovine BCG challenge model
L. Bifur1*, M. Vordermeier2, H. McMahan3, B. Villarreal-Ramos4;
1University of Oxford, Oxford, United Kingdom; 2Animal and Plant Health Agency, Weybridge, United Kingdom.

Bovine tuberculosis is a zoonotic disease affecting cattle and causing economic loss to farmers worldwide. Protection induced, by the currently only experimentally used BCG vaccine, varies between 0 to 80%. Lacking a correlate of protection every new vaccine candidate has to be tested in lengthy and expensive B. bovis challenge experiments. Methods that would allow for a faster and cheaper pre-selection of vaccine candidates and additionally improve welfare of experimental animals are needed. We have previously developed a bovine BCG challenge model in which animals are challenged intranodally with a non-pathogenic BCG strain. Improved protection results in reduced bacterial burden in the injected lymph nodes of protected cows compared to unprotected animals three weeks after challenge. The aim of this work was to analyse the sensitivity of the BCG challenge model and thus its ability to differentiate between different vaccine regimens resulting in varying degrees of protection. In order to do so we compared vaccination with BCG only to a heterologous Adenovirus Antigen BSA prime boost vaccine regimen which has previously been demonstrated to improve protection over BCG alone in pathogenic M. bovis challenge experiments. We show, that the BCG challenge model is able to measure improved protection of both vaccine regimens over unvaccinated control animals. However, the model is currently not sensitive enough to distinguish between the two different vaccine regimens. Nevertheless, we aim to make it possible to determine the neutralizing capability of vaccine sera before the intracerebral challenge.

P.D.3.02.03
Presentation of Hepatitis B virus antigens on human dendritic cells; hunting for novel immune therapy targets by Mass spectrometry
B. Roux1, K. Bezarostomi2, M. de Beijer3, P. Bastia4, A. Woltman5, J. Demmers5, S. Buschouw2;
1Erasmus MC Dept. of Gastroenterology and Hepatology, Rotterdam, Netherlands; 2Erasmus MC Dept. of Biochemistry, Rotterdam, Netherlands.

Approximately 350 million individuals worldwide have a chronic hepatitis B infection and as a consequence are at risk for developing hepatocellular carcinoma (HCC). In addition, 50% of all HCC cases arise in individuals infected with HBV, highlighting the contribution of chronic HBV infection to the high prevalence of HCC. Conventional therapies are not curative, are expensive and need to be taken life-long. We aim to design a therapeutic vaccine for the treatment of chronic HBV, that should boost or initiate immunological responses to critical Human leukocyte antigen (HLA) epitopes. To identify target epitopes we here loaded human monocyte derived dendritic cells with HBV antigens from different sources, isolated HLA I complexes and performed sensitive Mass spectrometry on eluted HLA-peptides. We thereby identify therapeutically relevant HBV-epitopes presented on professional antigen presentation cells. Currently we are expanding our methodology to HLA II and primary DCs.

P.D.3.02.04
Mixed mucosal-parenteral immunizations with the broadly conserved pathogenic Escherichia coli antigen SseI induce a robust mucosal and systemic immunity without affecting the murine intestinal microbiota
I. Naili1, J. Vivot1, B. C. Baudner1, A. Bernailler-Donadille1, M. Pizzol1, M. Desvaux1, G. Jubelin1, U. D’Oro2, C. Buonsanti2;
1GSK, Siena, Italy; 2Université Clermont Auvergne, INRA, Clermont-Ferrand, France.

E. coli can cause a vast range of intestinal (InPEC) and extraintestinal (ExPEC) diseases but only a very limited number of antibiotics still remains effective against this pathogen. A broad spectrum E. coli vaccine could be a promising alternative to prevent the burden of such diseases, while offering the potential for covering against several InPEC and ExPEC at once. SseI, Secreted and Surface-associated Lipoprotein of E. coli, is a widely distributed protein among InPEC and ExPEC. SseI functions ex vivo as a mucinase capable of degrading mucins and reaching the surface of mucus-producing epithelial cells. SseI was identified by reverse vaccination as a protective vaccine candidate against an ExPEC murine model of sepsis, and further shown to be cross-effective against other ExPEC and InPEC models of infection. In this study, we aimed to gain insight into the immune response to antigen SseI and identify an immunization strategy suited to generate robust mucosal and systemic immune responses. We showed, by analyzing T-cell and antibodies responses, that mice immunized with SseI via an intranasal prime followed by two intramuscular boosts developed an enhanced overall immune response compared to either intranasal-only or SseI and parenteral-only groups. Importantly, we also report that this regimen of immunization did not impact the richness of the murine gut microbiota. Mice had a comparable cecal microbial composition, whether immunized with SseI or PBS. Collectively, our findings further support the use of SseI in future vaccination strategies to effectively target both InPEC and ExPEC while not perturbing the resident gut microbiota.

P.D.3.02.05
Intranasal delivery of inactivated enterovirus D68 induces robust virus-specific humoral and cellular responses and confers protection against lethal viral challenge in mice
A. Chien1, Y. Lin1, P. Cheng2, B. Chiang3;
1Graduate Institute of Immunology, National Taiwan University, Taipei, Taiwan; 2Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan; 3Graduate Institute of Clinical Medicine College of Medicine, National Taiwan University, Taipei, Taiwan.

Intravenous delivery of inactivated enterovirus D68 induces robust virus-specific humoral and cellular responses and confers protection against lethal viral challenge in mice

C. Chien1, Y. Lin1, P. Cheng2, B. Chiang3;
1Graduate Institute of Immunology, National Taiwan University, Taipei, Taiwan; 2Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan; 3Graduate Institute of Clinical Medicine College of Medicine, National Taiwan University, Taipei, Taiwan.

Enterovirus D68 (EV-D68) has been recognized as a significant respiratory pathogen in children since its infections have remarkably increased worldwide during the past decade. In addition to severe respiratory tract infection, EV-D68 has been reported to associate with acute flaccid myelitis (AFM) in recent years. However, neither effective vaccines nor antiviral drugs are currently available to prevent its infections. Given that its infections have been demonstrated to depend on mucosal invasion, we sought to develop a potential mucosal vaccine against EV-D68.

Materials and Methods: C57BL/6 mice were intranasally immunized with the inactivated virus, and both humoral and cellular immune responses were assessed after the immunization. Sera collected from the vaccinated mice were further used in neutralization tests and passive protection assays.

Results: We found enhanced EV-D68-specific responses in C57BL/6 mice after the intranasal challenge, including the elevated antibody titers in nasal wash, saliva, bronchoalveolar fluid, feces, and sera, as well as the increased proliferative and IgG-producing ability of the restimulated spleocytes. We also confirmed the sera to have neutralizing and cross-neutralizing capability in human Rhadomyosarcoma (RD) cells. Furthermore, we found that anti-EV-D68 sera transferred before the intracerebral challenge lowered the incidence of paralysis and prolonged the survival of neonatal Institute of Cancer Research (ICR) mice. Tissue load analysis showed decreased viral levels in the spinal cord and limb muscles of the sera recipients, and the histopathological examination revealed reduced necrosis of hindlimbs of mice with the sera recipients.

Conclusion: Our data demonstrated the proof-of-concept for effective intranasal vaccination with inactivated EV-D68.
Adjuvants contribute to enhancing and shaping the vaccine immune response through different modes of action. Here, we profiled early biomarkers of adjuvancy after priming and investigated the impact of heterologous prime-boost approaches on the vaccine-specific immune responses. The modulation of primary T and B cell responses was characterized in mice primed with the mycobacterial antigen H56 and different adjuvants. Combinations of adjuvanted H56 priming with homologous or heterologous boosting, were also analysed. Vaccine formulations containing the liposome system CaPo1 or a squalene-based adjuvant elicited a primary CD4+ T cell response skewed to Th1/Th7 and Th1/Th2, respectively. Induction of short-lived plasma cells and early H56-specific IgG were observed mainly in mice immunized with CpG or the squalene-based adjuvant, while all tested adjuvants promoted the germinal centre reaction but with different magnitude. Mice primed with CaPo1, but not antigen alone, and boosted with homologous or heterologous formulations, showed a strong secondary CD4+ T cell response.

Computational analysis performed with the FlowSim software (c package) allowed clustering of antigen-specific T helper cells in different polyfunctional subsets producing combinations of TNFα, IL-2 and IFN-γ, that were observed only in mice primed with the adjuvant-boosted formulation, regardless of the booster formulation used. These results show that the immunological activity of different adjuvants can be characterised profiling early biomarkers after immunization and highlight the crucial role of the adjuvant in generating primary immune responses that can be recalled by boosting. These data could give an important contribution to the rational development of heterologous prime-boost vaccine immunization protocols.

**Modulation of primary immune response by different adjuvants to design heterologous prime-boost combinations**

A. Ciabattini, E. Petroni, F. Forinaz, S. Lucchesi, G. Pastore, F. Santorei, R. Andersson, G. Pozzi, D. Medaglini, 1University of Siena, Siena, Italy, 2Statens Serum Institute, Copenhagen, Denmark.

**Immunogenicity of Leishmania extracellular vesicles in combination with CpG ODN as a vaccine against cutaneous Leishmaniasis**

B. Gepkin, I. C. Ayanoglu, E. M. Ipkoğlu, G. Uş, M. Gürsel

1Department of Biological Sciences, Middle East Technical University, Ankara, Turkey, 2Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey.

Introduction: Cutaneous Leishmaniasis (CL), also known as Aleppo sore is a neglected parasitic disease that presents as self-healing skin lesions or cause debilitating large chronic or recurring lesions. During 1990-2010, >46,000 new CL cases caused by 3 different Leishmania species have been identified in Turkey. Considering the vast number of refugees immigrating to Turkey from Syria where CL is highly endemic, a substantial increase in CL cases is anticipated in the near future. The absence of an available licensed vaccine coupled with the cost, toxicity and drug resistance associated with the pentavalent antimonials used for treatment, necessitates the development of an effective preventive vaccine. Herein, we explored the immune protective vaccine potential of Leishmania antigen-rich small vesicles (exosomes) secreted from parasites in combination with CpG ODN based vaccine adjuvants.

Materials & Methods: Soluble leishmania antigen (SLA) or parasite exosomes were isolated from L. major. For vaccination, Balb/c mice were immunized twice with: heat-killed parasites, SLA or exosomes as such or in combination with CpG ODN based vaccine adjuvants. Leishmania antigen specific IgG levels were quantified from sera by ELISA. Th1/Th2/Th17 responses elicited by vaccine formulations were measured antigen stimulated splenocytes using cytometric bead array (CBA).

Conclusion: The results suggest that SLA and exosomes are promising anti-Leishmania protective antigens and D-type CpG ODN is the most promising adjuvant.

**Pigs as translational model: vaccination by tattoo with DNA in nanoparticles**


1Faculty of Veterinary Medicine, Utrecht, Netherlands, 2Netherlands Cancer Institute, Amsterdam, Netherlands, 3MIRA institute for Biomedical Technology & Technical Medicine, Enschede, Netherlands, 4Academic Medical Center, Amsterdam, Netherlands.

DNA vaccination in the skin is an established vaccination method in small laboratory animals. Historically, mice have been used as a preclinical model for dermal vaccine delivery, but extrapolation to humans may be difficult. Since the resemblance between pig and human skin, we investigated whether pigs may be used as preclinical model for dermal vaccination. Nanoparticle pDNA formulations were selected based on transfection efficiency of ex vivo pig skin. A selection of nanoparticle pDNA formulations in combination with the naked pDNA vaccine was subsequently applied in vivo by tattooing. Uptake of vaccine encoded antigen by cells in the draining lymph node (dLN) was determined by flowcytometry. Finally, HA37 formulated Human Papilloma Virus (HPV) 16 E6E7 DNA vaccine, currently used in a human trial, and unformulated DNA vaccine were assessed for their ability to stimulate T cells. Formulation of pDNA in polyplex HA37 resulted in the highest dermal transfection efficiency in pigs in vivo. Although cells in the dLN were able to take up the antigen, high background in some of the sham vaccinated pigs complicated this analysis. Tattoo with either HA37 DNA or naked DNA increased the number of IFN+ cells in the blood which was observed from the second vaccination onwards. In conclusion, DNA vaccination in the skin of pigs results in local production of antigen, its uptake by cells in the dLN and the induction of IFN+ cells. This suggest that DNA vaccination in the skin of pigs is an efficient way to induce vaccine-specific T cell responses.

**Corpuscular carrier enhances immune response after conjunctival immunization with chlamydial outer membrane proteins**


1Medical University of Vienna, Vienna, Austria, 2Institute of Virology, Vaccines and Sera, Belgrade, Serbia.

Trachoma, caused by the bacterial Chlamydia trachomatis (CT), remains the world’s leading preventable infectious cause of blindness. Recent attempts to develop effective vaccines rely on modified chlamydial antigen delivery platforms. We therefore characterized immune responses after conjunctival immunization with a N-terminal portion (amino acid 1-893) of the chlamydial polymorphic membrane protein C (PmpC) and a major outer membrane protein (MOMP) expressed in Escherichia coli bacterial ghosts (BGs) in a pig model of conjunctival infection animals. Three immunizations were performed at six week intervals, and the IgG immune responses were evaluated. Animals were further infected with C. caviae (1×107 IFU/animal) two weeks after the last immunization and ocular pathology and chlamydial clearance were investigated. Antigen-specific IgG levels in sera yielded significantly increased levels in the group immunized with MOMP BGs compared to animals immunized with PmpC BGs. Furthermore, a decrease in intensity of the recurring lesions. During 1990-2010, >46,000 new CL cases caused by 3 different Leishmania species have been identified in Turkey. Considering the vast number of refugees immigrating to Turkey from Syria where CL is highly endemic, a substantial increase in CL cases is anticipated in the near future. The absence of an available licensed vaccine coupled with the cost, toxicity and drug resistance associated with the pentavalent antimonials used for treatment, necessitates the development of an effective preventive vaccine. Herein, we explored the immune protective vaccine potential of Leishmania antigen-rich small vesicles (exosomes) secreted from parasites in combination with CpG ODN based vaccine adjuvants.

Materials & Methods: Soluble leishmania antigen (SLA) or parasite exosomes were isolated from L. major. For vaccination, Balb/c mice were immunized twice with: heat-killed parasites, SLA or exosomes as such or in combination with CpG ODN based vaccine adjuvants. Leishmania antigen specific IgG levels were quantified from sera by ELISA. Th1/Th2/Th17 responses elicited by vaccine formulations were measured antigen stimulated splenocytes using cytometric bead array (CBA).

Conclusion: The results suggest that SLA and exosomes are promising anti-Leishmania protective antigens and D-type CpG ODN is the most promising adjuvant.

**Variane and dynamism of humoral responses in humans after a novel intranasal RSV subunit vaccine**


1Section of Infectious Diseases & Immunology, Imperial College London, London, United Kingdom, 2National Heart and Lung Division, Imperial College London, London, United Kingdom, 3Mucocis B.V., 4Bout Advocaten, Groningen and Virtuvax B.V., 5Department of Clinical Infection, Microbiology and Immunology, Institute of Infectious and Global Health, University of Liverpool, Liverpool, United Kingdom.

Respiratory syncytial virus (RSV) is a major cause of respiratory illness in both infants and the elderly but despite decades of research, no effective vaccine exists. We recently showed in human RSV infection challenges that mucosal IgA associated more closely with protection from infection than any other recognised correlate. Intranasal vaccination to induce both mucosal and systemic immunity could therefore offer preferential protection against RSV and other respiratory pathogens.

Here, we report immunological and cellular immunity data from a randomised controlled Phase 1 trial of an intranasal subunit vaccine comprised of the RSV F protein linked to a bactericidal-like particle (BLP). The vaccine was well tolerated and significantly induced antibody-secreting cells, which were associated with consistent increments in anti-F protein serum IgG. Nasal IgA responses displayed heterogeneity in timing and magnitude, but very large increments seen in those with lower pre-existing titres. However, serum antibodies were preferentially non-neutralising and antibodies directed against the pre-fusion F protein conformation were not detectable. In vitro culture of tonsilar mononuclear cells from adults and children with the vaccine induced anti-F protein antibodies in a dose-dependent manner and was associated with marked CD4+ and CD8+ T cell proliferation with production of IFN-γ, IL-2 and IL-22.

This novel intranasal BLP platform can stimulate both mucosal and systemic responses, with serum antibody and plasmablast response comparable to live attenuated virus vaccines. The added advantages of subunit vaccine manufacturing and stability suggest that this vaccine can be a candidate for further optimisation.
P.D3.02.13 Immunogenic responses to improve anti-carbohydrate vaccines against bacterial pneumonia

M. Gomez Pera-Bordas, M. Rea

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

459
Experimental identification of conserved CD8 T epitopes, was studied by IFN-γ ELISPOT assays using peripheral mononuclear cells (PBMC) from typed subjects exposed to synthetic peptides, consisting of predicted HRV-specific CD8 T epitopes. We have characterised 29 candidate epitopes derived from the computational analysis (25 of RV14 A and 14 of HRV C) and 20 of them obtained a positive result in the analysis of release of IFN by ELISPOT. We verified peptide-binding to HLA-A0201 of ELISPOT positive responses using TAP-deficient T2 cell line binding assays, which has led to the experimental identification of two Rhinovirus epitopes (VEKGIPQ and GLEEPDLNTAGFPYV) restricted by this allele that could potentially serve for the development of a vaccine.

P.D3.02.19
Optimization of HIV-1 DNA vaccine by comparing Minicircle and conventional vectors
H. Huang, W. Yikchun, C. Zhiwei, C. Samantha, C. Moxem; AIDS institute, Hong Kong, China.
DNA-based vaccine has become a promising vaccine vector because of its good safety profile and high immunogenicity. However, many current DNA vaccines only support short-term antigen expression and have low protective efficacy. Part of the reason is due to their large sizes and the presence of bacterial backbone sequences. Minicircle DNA vector is a next generation circular DNA vector that mostly consists of a small expression cassette, without the unnecessary bacterial backbone. Several studies have reported that minicircle vector enhances expression level and duration of inserted transgenes. In this study, we examined the efficacy of minicircle vector in comparison to conventional pVAX plasmid as a vaccine against a HIV antigen in mouse models. The recombinant antigen focused in this study consisted of a soluble problem death 1 (PD-1) molecule fused to a mosaic HIV-Gag-p41 antigen that was designed in silico based on the major circulating HIV sequences in China. The soluble PD-1 molecule allows effective antigen targeting to dendritic cells for antigen presentation. We firstly confirmed the long-term antigen expression in mice treated with minicircle DNA vaccine intramuscularly with electroporation. Mice immunized with the minicircle vaccine induced a trend of higher CD8+ in the spleen, lung and genital tract than those injected with an equal mass of the full-length pVAX vaccine during both acute and memory phases. More importantly, the minicircle vaccine conferred a better protection in mice against intercostal challenge with malignant mesothelioma AB1 cells expressing HIV-Gag antigen. Collectively, our results demonstrate that minicircle vaccine is a favorable vaccine vector for inducing strong protective T cell immunity.

P.D3.02.20
Autoreactive potential of universal influenza vaccines
M. R. Pillai, T. Chang, J. Crawford, R. Keating, C. Lewis, J. Labombardé, Q. Li, P. G. Thomas, M. A. McGarrill; 1Division of Infectious Diseases, University of California, Los Angeles, United States, 2UT Southwestern, Dallas, United States.
A universal influenza vaccine could save millions of lives in the event of a deadly pandemic. It is not clear why antibodies specific for conserved regions of influenza viruses are so rare. One possibility is that these antibodies have a higher potential to cross-react to self-proteins, and therefore B cells that generate these antibodies are deleted through tolerance mechanisms. In support of this, infections and vaccinations with the 2009 H1N1 pandemic strain induced more antibodies that were cross-reactive against multiple influenza strains than were induced by previous seasonal strains. However, they were also associated with a higher risk of autoimmune disorders, including narcolepsy and Guillain-Barre syndrome. Therefore, we examined whether cross-reactive influenza antibodies had a higher potential to be autoreactive than antibodies specific for one subtype of influenza. We previously demonstrated that H3N2-vaccinated mice treated with a low dose of rapamycin had more cross-reactive influenza antibodies and were better protected against subsequent lethal infections of multiple subtypes. Thus, we utilized rapamycin to increase the frequency of influenza cross-reactive antibodies, and tested whether these antibodies were more reactive to self-proteins than strain-specific antibodies. We found that mice with increased levels of cross-reactive influenza antibodies also had more IgM antibodies specific for self-antigens. Although the increase in autoreactive IgM antibodies was transient, it correlated with increased susceptibility to disease in mouse models of multiple sclerosis and Guillain-Barre Syndrome. Together, our results suggest that influenza cross-reactive antibodies have the potential to be autoreactive. These data have important implications for developing universal influenza vaccines designed to generate durable influenza cross-reactive antibodies.

P.D3.02.21
PHASE I, DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED SAFETY AND IMMUNOGENICITY TRIAL OF REACTIVATION OF PERTUSSIS TOXIN IMMUNITY WITH AN INVESTIGATIONAL EPICUTANEOUS PATCH IN HEALTHY ADULTS.
Background: Novel immunization strategies against pertussis are needed as immunity induced by acellular vaccines can be short-lived. Additionally, vaccination with needle-less and adjuvant-free patches can prepare the target population benefiting from immunization. Methods: A Phase I, double-blind placebo-controlled randomized trial assessed safety and immunogenicity of an epicutaneous patch, Viaskin, administering recombinant pertussis toxin (PT), in healthy adult (last PT-vaccinated>10 years). Viaskin patches of PT 25µg, 50µg or placebo were administered at day 0 and 42, applied directly on the skin (n=25, 25, 10) or after controlled epidermal laser-based skin preparation (n=5, 25, 12). The primary outcome was safety of Viaskin PT. Antibody responses were assessed at day 14, 28, 42 and after administration of Boostrix®dTpa on day 70. Results: Baseline characteristics were similar among groups. Safety and tolerability profiles were favorable. Application directly on skin generated no detectable immunogenicity signal.In the group treated with Viaskin following skin preparation, D42 PT-IgG Geometric Mean Concentrations (GMCs) were significantly higher compared to placebo (p<0.001): Viaskin PT25: 33.24 IU/ml (95% CI, 9.59; 115.23), Viaskin PT50 (57.00 IU/ml (95% CI, 41.39; 78.51), placebo (4.03 IU/ml (95% CI, 2.56; 6.37). D42 seroconversion rates were significantly higher with Viaskin PT25 and PT50 vs. placebo (80% and 88% vs. 0%, p<0.002 and p<0.001, respectively). One-month after Boostrix®dTpa, PT-IgG antibody levels were similar in all groups.Conclusions: Viaskin PT applied after epidermal skin preparation resulted in favorable safety and immunogenicity: anti-PT booster responses were comparable to those elicited by Boostrix®dTpa.Clinical Trial Registration: NCT 03053570

P.D3.02.22
A replication-incompetent adenoviral vector-based HSV-2 vaccine induces strong humoral responses and protects mice against lethal HSV-2 challenge
Herpes Simplex Virus-2 (HSV-2) is the leading cause of genital herpes and a major global health problem. HSV-2 resides in nerve cells and can reactivate sub-clinically or cause genital blisters. Several prophylactic vaccines against this lifelong disease have been studied and failed in the clinic to show efficacy against the infection. Therefore, the development of an effective HSV-2 vaccine is urgently needed. Replication-incompetent adenoviral vectors are a very promising vaccine platform and they have shown to be safe and capable of inducing both humoral and cellular immune responses in animal models and in humans. We have generated an adenoviral vector-based HSV-2 vaccine. Immunogenicity and efficacy of the vaccine were evaluated in an HSV-2 challenge model in mice. Virus neutralization and ELISA titers were measured in serum before challenge. Viral titers, clinical scoring and survival rate were monitored after intragastrical challenge with 200 LD50 HSV-2 strain virus. The HSV-2 vaccine candidate induced comparable high virus neutralizing antibody titers, both after prime and prime-boost immunization. Both vaccine regimens induced complete protection against lethal challenge with the wild type HSV-2 strain virus. We are currently investigating HSV-2 latency in dorsal root ganglia isolated from the infected mice. Additionally, experiments are conducted to further characterize the humoral response by measuring IgG subclasses and antibody dependent cellular cytotoxicity. Cellular responses will be measured by IFN-γ ELISPOT and intracellular cytokine staining. The results from this study strongly encourage us to investigate more thoroughly this promising vaccine platform against HSV-2.
**POSTER PRESENTATIONS**

**P.D3.02.23**

**Education of Stem Cells by BCG: An innovative Approach in TB Vaccine Development**


**Introduction**

BCG is still the only available vaccine against TB, but prevents only the disseminated forms of the disease in early childhood. The efficacy of BCG against pulmonary TB in adults ranges from 0.80% to 8.0%. While control of TB requires T-cells to prevent disease progression, clinical trials of T-cell-targeting vaccines have failed to demonstrate protection against Mycobacterium tuberculosis (MtB). We therefore hypothesize that a protective mechanism afforded by BCG in adults is mainly dependent on macrophages which are one of the first immune cells to encounter MtB upon infection. However, considering the nature of monocyte/macrophage differentiation and their relatively short lifespan, we hypothesize that the access of BCG to the bone marrow (BM) will educate the hematopoietic stem cells (HSCs) to subsequently generate protective innate immunity.

**Methods & Results**

Using mouse models, we demonstrated that following intravenous but not subcutaneous BCG vaccination, the bacteria access the BM. The presence of BCG in the BM significantly increased the numbers of lineage-c-KItSca1 HSCs as well as multi-potent progenitors, and led to the generation of epigenetically modified macrophages/macrophages. By using parabiotic and chimeric mice as well as adoptive transfer approaches, we demonstrate that these educated macrophages/macrophages provide sustainable protection against MtB infection in vivo.

**Conclusions**

Our findings demonstrate that access of BCG into the BM is critical for generating a unique set of educated macrophages/macrophages that are protective against virulent MtB infection. Reprogramming of HSCs thus may provide an innovative approach in vaccine development.

This work was supported by CHF, DFG and FQRS.

**P.D3.03 Novel approaches to vaccinology - Part 3**

**P.D3.03.01**

**Bordetella pertussis challenge fails to recall vaccine- and pre-exposure-induced circulating memory B cells**

M. Ballesta1,2, B. Mastelic-Gavillet1,2,3, P. Fontanona1,2,3, F. Auderset1,2,3, P. Lambert1,2, C. Sigrist1,2,3.

1Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland, 2World Health Organization Collaborating Center for Vaccine Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland, and 3Center for Vaccinology, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland.

**Introduction**

Despite decades of vaccine prevention, pertussis continues to occur and its prevalence is increasing. Clinical trials have demonstrated the efficacy of the current acellular vaccines (aP), however primo-infection still confers a better and more sustained protection. In humans, vaccine-induced antibodies rapidly wane after immunization, suggesting the failure to induce long-lived plasma cells. This contrast with murine data, in which antibody titres only slowly decline, in correlation with the persistence of plasma cells in the bone marrow. To circumvent the issue of antibody persistence in mice, we developed an adoptive transfer model in which memory immune cells induced by primo-infection or aP vaccination and pre-exposure are transferred to naïve recipients prior to booster immunization or intranasal challenge with Bordetella pertussis (BP). This allows us to assess recall responses in the absence of circulating serum antibodies, mimicking the situation in humans.

Using this model, we found that BP challenge recalls both vaccine- and pre-exposure-induced B cell memory much slower than a vaccine booster. As in humans, pre-exposure to BP primed the protection than vaccine priming, efficiently initiating bacterial clearance in the first week post-challenge. This implies (i) that the delayed reactivation of memory B cells by BP contributes to the lack of protection against BP, and (ii) that other key protective memory cells are induced by primo-infection but not aP vaccines.

**P.D3.03.02**

**A prime with naked DNA improves the immune response induced by a modified live vaccine against Porcine Reproductive and Respiratory Syndrome Virus**

I. Bernelin-Cottet1, C. Urien1, F. Blanc1, V. Jakab2, E. Bordet1, C. Barc1, V. Contreras1, N. Bertho1, C. Barrier-Quer1, E. Studarab1, A. Brunsvik Fredrikson1, H. Nauwynck1, I. Schwartz-Cornill1;

1VIM-INRA-Université Paris-Saclay, Jouy-en-Josas, France, 2GABI-INRA-AgroParisTech-Université Paris-Saclay, Jouy-en-Josas, France, 3Vaccine Formulation Laboratory, Department of Biochemistry, University of Lausanne, Epalinges, Switzerland, 4INRA, UE1277, Plate-Forme d’Immunologie Expérimentale, PFIE, Jouy-en-Josas, France, 5CEA-Université Paris Sud-ÎnesR, U1184 + Immunology of viral infections and auto immune diseases », IDAMRT department, IBL, Fontenay-aux-Roses et Armenin-Bicêtre, France, 6Vaccine AS, Oslo, Norway.

The Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) induces reproductive disorders in sows and respiratory illnesses in growing piglets and is considered as the main pathogenic agent responsible for economic losses in the porcine industry worldwide. Modified live PRRSV vaccines (MLV) are by far the most efficient vaccine types but they are mainly protective against homologous strains and they may reverse to pathogenicity upon residual replication. We aimed at evaluating DNA vaccines as stand-alone vaccines or used as priming to improve the MLV efficacy and safety. Our DNA PRRSV vaccines encode B and T antigens from a European subtype 1 strain which are relatively conserved across strains and which are expressed in a native form or in the form of vaccinodies targeted to the endocytic receptor CD11c expressed by dendritic cells. When delivered in skin with surface electroporation, the DNA vaccines induced antibody and T cell responses which were not promoted by antigen targeting to dendritic cells. Vaccination alone vaccines or used as priming to improve the MLV efficacy and safety. Our DNA PRRSV vaccines encode B and T antigens from a European subtype 1 strain which are relatively conserved across strains and which are expressed in a native form or in the form of vaccinodies targeted to the endocytic receptor CD11c expressed by dendritic cells. When delivered in skin with surface electroporation, the DNA vaccines induced antibody and T cell responses which were not promoted by antigen targeting to dendritic cells. Vaccination alone vaccines or used as priming to improve the MLV efficacy and safety. 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Abstracts of the 5
462
POSTER PRESENTATIONS
Clinical symptoms of dengue virus (DENV) infection, the most prevalent arthropod-borne viral disease, range from classical mild dengue fever to severe, life-threatening dengue shock syndrome. However, most DENV infections cause few or no symptoms. Asymptomatic DENV-infected patients provide a unique opportunity to decipher the host immune responses leading to virus elimination without negative impact on an individual's health. We used an integrated approach of transcriptional profiling and immunological analysis to characterize the consequences of strictly asymptomatic dengue infections in humans. Whereas infections in vaccinated and non-vaccinated individuals share many features, we observed several differences in their immune responses. In particular, early activation of T cell populations, comprising upregulation of T cell activation markers and expression of transcripts related to the innate immune system, and the time it took for vaccine-induced CD8+ T cell responses to develop, were similar between symptomatic and asymptomatic individuals. Clinical and laboratory-based studies have demonstrated that asymptomatic infection with DENV is associated with increased activation of the adaptive immune response and proper control mechanisms, leading to elimination of viral infection without excessive immune activation, with implications for novel vaccine development strategies.

P.D3.03.06
Age-dependent pre-vaccination immunity affects the immunogenicity of Varicella Zoster vaccine in middle-aged adults
M. van der Heiden1,2, L. G. H. de Rand1, M. C. van Zeijl1, G. A. Berbers1, A. M. Boots1, A. Buismans1
1National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, 2University of Groningen, Groningen, Netherlands. The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, -Erasmus MC, Rotterdam, Netherlands, 1Central Clinical School, Monash University and Alfred Hospital, Melbourne, Australia.
Background The Herpes Zoster disease increases with age. Unfortunately, vaccine strategies to harness ZV-specific cell-mediated immunity (CMI) demonstrate low effectiveness in the elderly, due to immune aging. To improve vaccine performance, we investigated immune responses after ZV vaccination (Zostavax) in Dutch middle-aged adults (N=53, 50-65 years of age). Methods Blood samples were taken pre-, 14 days, 28 days, and 1 year post-vaccination. ZV-specific IFN-γ-producing cells were measured by Elispot, activated T-cells by flow cytometry, IgG and IgA antibody levels by fluorescent bead-based multiplex immunassays, and whole blood cellular kinetics by TruCOUNT analysis. Results Robust short-term ZV-specific immune responses were observed post-vaccination. Remarkably, long-term ZV-specific IFN-γ responses in the elderly were comparable to those observed in younger adults. Conclusion These results suggest that adults in their early 50s, who showed a high CDA/CDB T-cell ratio and low ZV-specific CMI, benefit from ZV vaccination, also shown by a ZV-specific IgA response at day 14 post-vaccination. This provides important knowledge for strategies to strengthen ZV-specific immunity before reaching old age.

P.D3.03.07
Vaccinated induced immune responses in Gabonese infants
M. Esen1,2, J. Flügger1,2, J. Honkhepedjé1,3, E. Askani1,2,4, M. Massinga Loembe1,2,3, S. Brückner1, M. Duoss1, J. Strunk2, B. Mordmüller1,3, S. T. A. Gandjäi2,3, L. S. Blyth1,2, P. G. Kremsner1,2,5, A. A. Adenekan1,2,3
1Institut für Tropenmedizin, Tübingen, Germany, 2Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon, 3German Center for Infection Research (DZIF), Tübingen, Germany, 4Université des Sciences de la Santé, Libreville, Gabon.
Helminth infections are common in Sub-Saharan Africa. It is generally recognized that infected individuals show a bias towards a Th2 immune response. Most vulnerable to helminth infection are children and pregnant women. The consequences of parasitic infection during pregnancy for the mother and particularly for the fetus can be severe and may include late effects on immune response during acute infection and vaccination. From February 2014 until September 2016 we conducted a study to investigate the influence of maternal parasitic infection during pregnancy on the immune system of their infants in Lambaréné, Gabon and surroundings (ClinicalTrials.gov Identifier: NCT02714348). Here we present the data on immune responses to vaccines given within the expanded program on immunization (EPI) in 123 infants born to helminth infected and non-infected mothers. Helminth infection was diagnosed microscopically by the Kato-Katz method. Antibody titers to different vaccines (diphtheria, whooping cough, tetanus, poliomyelitis, hepatitis B and Haemophilus influenzae A) were measured using commercial and validated ELISA kits. Our data show that infection with helminths is still common in pregnant women in Gabon but has only subtle effects on infants’ immune responses to vaccines given as part of the EPI. Funding: This work was supported by the German Federal Ministry of Education and Research [01 KA 1005].

P.D3.03.08
SapM mutation to improve BCG vaccine efficacy: impact on vaccine clearance and quality of adaptive immune response
N. Festjen1, K. Vandewalle1, E. Houyoux1, E. Piets2, D. Vanderschaeghe1, K. Borgers1, A. Van Hecke1, P. Tiele1, N. Callaerts2
1VIB-UGent, Zwijnaarde, Belgium.
2The bovis Bacille Calmette-Guérin (BCG) vaccine shows variable efficacy in protection against adult tuberculosis (TB). Earlier, we have described a BCG mutant with a transposon insertion in the gene coding for the secreted acid phosphatase SapM, which led to enhanced long-term survival of vaccinated mice challenged with TB infection. We have now further characterized the genome and transcriptome of this sapM-Tn mutant versus parental BCG Pasteur. Moreover, we investigated the clearance of this improved vaccine strain from the immunization site, and the evolved immune response upon vaccination. Our findings strongly suggest that a more effective innate immune control over the vaccine bacteria leads to a more modest primary expansion of IFNγ Th1 and Th17 cells. This correlates with an improved control of BCG sapM-Tn bacterial loads compared to WT BCG following vaccination.

P.D3.03.09
DNA vaccination with APC-targeted hemagglutinin for prevention of influenza pandemics
G. Grodeland1,2, T. Andersen1,2,3, A. B. Fredriksen1,2, B. Bogen1,3,4,5, T. A. van der Heiden1,2,3, A. M. Boots1,2, A. Buisman1,2, A. G. Cattoglio1,2, C. Joly1,4, S. Brückner1,2, A. Tawfik1,4,5
1National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, 2Institut Pasteur Cambodia/Institut Pasteur International Network, Phnom Penh, Cambodia, 3INRA Sozay, Pelleu, France.
Clinical symptoms of dengue virus (DENV) infection, the most prevalent arthropod-borne viral disease, range from classical mild dengue fever to severe, life-threatening dengue shock syndrome. However, most DENV infections cause few or no symptoms. Asymptomatic DENV-infected patients provide a unique opportunity to decipher the host immune responses leading to virus elimination without negative impact on an individual’s health. We used an integrated approach of transcriptional profiling and immunological analysis to characterize the consequences of strictly asymptomatic dengue infections in humans. Whereas infections in vaccinated and non-vaccinated individuals share many features, we observed several differences in their immune responses. In particular, early activation of T cell populations, comprising upregulation of T cell activation markers and expression of transcripts related to the innate immune system, and the time it took for vaccine-induced CD8+ T cell responses to develop, were similar between symptomatic and asymptomatic individuals. Clinical and laboratory-based studies have demonstrated that asymptomatic infection with DENV is associated with increased activation of the adaptive immune response and proper control mechanisms, leading to elimination of viral infection without excessive immune activation, with implications for novel vaccine development strategies.

P.D3.03.10
Characterization of early responses in blood skin and lymph node compartments induced by intradermal injection of Modified Vaccinia Ankara
P. Rosenbaum1,2, N. Tchitchek1, C. Joly1,3, L. Stimmer1, H. Hocnin1, N. Deredieu-Bouquet1, A. Beignier1,4, C. Chapot2,4, L. Pelet3, R. Le Grand1, F. Martinou1,5,6
1Inmunology of Viral Infections and Autoimmune Diseases, IDIMT Department, CEA – Université Paris Sud 21 – INSERM U1184, Fontenay-aux-Roses, France, 2CEA – INSERM, IRGCM, USMBF, Fontenay-aux-Roses, France, 3CEA – INSERM, ILRIR, Fontenay-aux-Roses, France, 4CEA – INSERM, IRGCM, USMBF, Fontenay-aux-Roses, France, 5CEA – INSERM, U955, Henri Mondor Hospital, University of Paris East, Créteil, France, 6CEA – INSERM, IRGCM, USMBF, Fontenay-aux-Roses, France.
Vaccine design approaches would be greatly facilitated by a better understanding of the early immune changes, and those that occur at the site of injection, inducing a durable and oriented protective response. We were characterized in details very early infection and host response events after the intradermal administration of the modified vaccinia virus Ankara as a live attenuated vaccine model in non-human primates. We performed in vivo imaging, histology, flow cytometry, multiplex cytokine, and transcriptomic and analyzed data using tools derived from systems biology, such as co-expression networks.

We showed a strong early local and systemic inflammatory response that peaked at 24 h, which was then progressively replaced by an adaptive response during the installation of the host response to the vaccine. Granulocytes, macrophages, and mononuclear cells were massively recruited during the local innate response in association with local productions of GM-CSF, IL-1β, MIP-1α, MIP-1β, and TNFα. We also observed a rapid and transient granulocyte recruitment and the release of IL-6 and IL-10, followed by a persistent phase involving inflammatory monocytes. This systemic inflammation was confirmed by molecular signatures, such as up-regulations of IL-6 and TNF pathways and acute phase response signaling. Such comprehensive approaches improve our understanding of the spatio-temporal orchestration of vaccine-elicted immune response, in a live attenuated vaccine model, and thus contribute to rational vaccine development.
POSTER PRESENTATIONS

P.D3.03.12
Tannic acid-modified silver nanoparticles as novel adjuvants in herpes virus infection
P. Ornłowski, M. Tomaszewska, K. Raniszew-Soliwoda, G. Celichowski, J. Grobelny, M. Krzyzowska;
1Military Institute of Hygiene and Epidemiology, Warsaw, Poland, 2Faculty of Chemistry, University of Lodz, Lodz, Poland.

Silver nanoparticles (AgNPs) are promising new antimicrobial agents against a wide range of skin and mucosal pathogens. We have previously shown that tannic acid modified silver nanoparticles (Ta-AgNPs) consist a promising microbicide against genital herpes. The aim of this study was to study the ability of Ta-AgNPs to induce DCs activation in the presence of HSV-2 antigens when used at non-toxic doses. Additionally, we tested ability of Ta-AgNPs to induce efficient anti-viral immunity in vivo using mouse genital herpes model.

Preparation of HSV-2 treated with nanoparticles (Ta-AgNPs HSV-2) were used to investigate HSV-2 antigen uptake, activation markers and cytokine production by JAWS II dendritic cell line. We also accessed proliferation and activation of HSV-2 specific T cells by DCs treated with Ta-AgNP-HSV-2. Our results showed that Ta-AgNPs were potent stimulators of DCs maturation and helped to internalise viral antigens. Ta-AgNPs HSV-2 also helped to overcome inhibition of DCs maturation by live or inactivated virus through up-regulation of activation markers and cytokine production.

P.D3.03.13
Circulating T follicular helper cells and immune response induced by influenza vaccine in children with acute lymphoblastic leukemia during maintenance therapy
N. Le Corre1, C. R. Martínez-Valdenoño1, F. Barring1, M. Contreras1, M. Vidal1, R. Moreno1, X. Clavería1, P. Contreras1, L. Huneman1, R. Alarcón1, T. García1, R. Rathonasinho1, R. Medrón1, M. Ferreus1;
1Bartolomé de las Casas Infectious, Infecciosa e Immunológica Pediatrica, Pontificia Universidad Católica de Chile, Santiago, Chile, 2Laboratorio de Infectología y Virología Molecular, Red Salud UC-Christus, Santiago, Chile, 3Unidad Oncología División Pediatría, Pontificia Universidad Católica de Chile, Santiago, Chile, 4Unidad Hemato-Oncología Pediatrica Complejo Asistencial Dr. Sátero del Río, Santiago, Chile.

Vaccine immune response is impaired in immunocompromised patients. Follicular helper-T-lymphocytes (Tfh) are essential for high-affinity and long-lasting humoral response. We evaluate the role of Tfh in immune response induced by influenza vaccine in children with acute lymphoblastic leukemia (ALL). Children with ALL in maintenance therapy and a control group of healthy children were included. Blood samples were taken on the day of vaccination (D0), and on day 28 (D28). The humoral response and frequency of Tfh were evaluated. Twenty-four children with ALL and 8 healthy children included: 66,7 and 38% were women, median age of 5 years old in both. A 33,3% (8/24) of patients and 63%(5/8) of controls were seroprotected at D28. Seroprotected children were significantly older (10 years) than non-protected children at D28 (3.6 years, p=0.004). During follow-up, three children had influenza infection. An increase of percentage of Tfh cells from D0 to D28 was observed in both groups, but only significantly in ALL (mean-ALL, D0-28:18-23% (p=0.003) and mean-controls, D0-28:22-26%). Comparing seroprotected versus non-seroprotected children no differences were found in Tfh cell at D0 or D28. The increase of percentage of Tfh cells from D0 to D28 observed in both groups, was significant only non-seroprotected subject (mean-seroprotected, D0-28:21-24%and mean-non-seroprotected, D0-28:18-24% p=0.004). Children with ALL and a low percentage of Tfh compared to healthy children but children who received vaccination had an increase of Tfh cells. We did not found association between percentage of Tfh cells and seroprotection at D28. It should be evaluated if the lack of humoral response is associated to Tfh dysfunction. Fondedyt-1150970.

P.D3.03.14
An Immunogenic Epitope Chimeric Protein of HAdV for Antibodies Detection as well as Immunity Analysis
Y. Li, Y. Li, X. Wang, J. Li, R. Zhao, W. Shen, W. Zeng, S. Liu, X. Li, Y. Lin; Huadong Research Institute for Medicine and Biotechniques, Nanjing, China.

1META NAME="author" CONTENT="Microsoft Office II" /> To construct and express a chimeric protein of immunogenic epitopes from five types of human adenoviruses(HAdV), type 3, 7, 11, 14 and 55, and identify its immunoactivity.

The amino acid sequences of hexons from the five types of HAdV were analyzed respectively by using the chimeric protein, and the anthrombic epitopes with strong antigenicity were screened. The selected immunogenic epitopes were linked together with Gly-Gly-Ser for constructing a chimeric protein of immunogenic epitopes. The DNA of the chimeric protein was synthesized chemically, and cloned into plasmid PET-28a (+) for expressing the chimeric protein. The chimeric protein was purified by Ni-NTA resin and used as an antigen to immunize Balb/c mice, the antisera was prepared. The antigenicity and immunogenicity of the chimeric protein were detected by ELISA. The chimeric protein was expressed and produced efficiently in the monolayer culture of CHO cells. The titters of antiserum from the mice immunized four times with the chimeric protein reached to 1:320,000, and the ELISA results confirmed that the chimeric protein has strong antigenicity and immunogenicity. The expressed chimeric protein of immunogenic epitopes from the five types of HAdV laid down for developing vaccine and diagnostic reagents.

Keywords: human adenovirus; chimeric protein; antigenicity; immunogenicity

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P.D3.03.15
Repeated mycobacteria vaccination in mice induces myeloid-derived suppressor cell killing of splenic dendritic cells via INOS-dependent NO production
E. Ribeiro1, J. Eckert1, A. Beilhac2, N. Du Plessis3, G. Wold1, U. Schleicher2, U. Ritter3, M. B. Lutz2; 1University of Würzburg, Würzburg, Germany, 2Military Institute of Hygiene and Epidemiology, Warsaw, Poland, 3University of Erlangen, Erlangen, Germany, 4University of Regensburg, Regensburg, Germany.

Myeloid-derived suppressor cells (MDSCs) accumulate in patients with tuberculosis (TB) and a vaccine based on Mycobacterium tuberculosis (MtB) is lacking. From this, we hypothesized that MtB-based vaccines may induce MDSCs impairing vaccination success. Our data indicate that in vitro, bone marrow-derived resting MDSC (R-MDSC) stimulation with heat-killed MtB resulting in the production of NO, directly suppressing T cells and inducing bone marrow-derived dendritic cell (BM-DC) apoptosis. The killing was NO dependent since blocking of INOS reverted the effect. In vivo, repeated immunization of mice with Complete Freund’s Adjuvant (CFA) containing MtB but not incomplete Freund’s Adjuvant (IFA) lacking the MtB component induced activated MDSCs (A-MDSCs) in the spleen. Myeloid cells isolated from spleens of CFA/IFA injected mice but not single CFA or IFA/IFA injected mice suppressed CD4+ and CD8+ T cell proliferation in a nitric oxide (NO) dependent manner. The accumulation of iNOS-1/CD11b+ MDSCs was restricted to the splenic red pulp and bridging channels. Short term microbial challenge of mice in vivo induced infiltration of INOS+ A-MDSCs after 6h into the white pulp resulting in conventional DCs (cDCs) and plasmacytoid DCs (pDCs) apoptosis in the T cell area of the white pulp after 24h. DC apoptosis was not observed after microbial challenge alone and was reduced in NO2− mice. In contrast, apoptosis of T cells was not observed and macrophage killing occurred but was independent of NO alone. Together, our data indicate that MtB vaccines induced and activated MDSCs in spleens of mice leading to NO-dependent DC killing.

P.D3.03.16
Immun profile driven by a novel tuberculosis nanovaccine correlates with protection against Mycobacterium tuberculosis infection
A. Martínez-Pérez1, A. Igra1, O. Estévez-Martínez1, C. M. Ferrera1, A. G. Castro4, E. Torrado1, A. González-Fernández2; 1Immunology, Biomedical Research Centre (CIBIO), 2Singular Centre of Research, 3Galicia Sur Health Research Institute (IISGS), 4University of Vigo, Vigo, Spain, 4Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, and 5PT Government Associate Laboratory (CVS/385), Braga, Portugal.

Introduction: The mechanisms underlying protection against Mycobacterium tuberculosis (MtB) infection remain unclear. Prime vaccination with BCG following by boosting actions with novel vaccines has emerged as a promising strategy. In this regard, a new vaccine composed by a nanoparticle and a fusion protein containing three MtB antigens and administrated intranasally, has been shown to enhance protection against MtB when compared to BCG alone. In this study, we aimed at defining the immune cellular and molecular profile generated by this vaccine with the ultimate goal of identifying correlates of protection generated by this vaccine with the ultimate goal of identifying correlates of protection generated by this vaccine.

Materials and methods: Mice were vaccinated with BCG following by two boosts two weeks apart with two different nano-vaccines. Two weeks after the last boost, mice were sacrificed and lung and spleen cells analyzed by flow cytometry and RNA-Sequencing.

Results: We found different protection levels induced by the tested nanovaccines. Interestingly, the level of protection correlated with the polyfunctionality of the Ag-specific CD4+ T cell response and composition of the lung immune infiltrates. Crucially, gene expression analysis revealed a unique profile of differentially expressed genes in protected mice.

Conclusions: The results obtained in this study offer new insights that may be useful in the design of novel and more efficient vaccines to tuberculosis.
P.D.03.01.1
Targeting of influenza viral epitopes to antigen presenting cells by genetically engineered chimeric molecules in humanized NSG transfer model

M. Mihaylova1, I. Ivanova1, I. Manoylov1, D. Makatso1, S. Loloa2, M. Nikolova3, A. Mamalaki4, J. Preclí5, A. Tchorbary6,7; 1Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; 2Hellenic Pasteur Institute, Ampelokipi, Athens, Greece; 3Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria; 4National Reference Laboratory of Immunology, NCIPD, Sofia, Bulgaria; 5Immunology Research Group, Hungarian Academy of Sciences, Budapest, Hungary; 6National Institute of Immunology, Sofia, Bulgaria.

Introduction: Anti-viral DNA vaccines are a novel strategy in the vaccine-development field, which consists in the administration of expression vectors coding viral antigen sequences inserted into the host’s cells. It has been shown that FcγRI on human monocytes enhances antigen presentation in vivo. Targeting of conserved viral epitopes by antibody fragments specific to activating cell surface co-receptor molecules on antigen-presenting cells could be an alternative approach for inducing protective immunity. Materials and Methods: Genetic engineering, signal transduction, cell transfection, flow cytometry, generation of humanized NOD/SCID model, ELISPOT, ELISA and cytotoxicity assays; Results: Various DNA constructs, encoding a scFv fragment from mouse anti-human FcγRI monoclonal antibody, coupled to a sequence, encoding a T- and B-cell epitope-containing influenza A virus hemagglutinin intersubunit peptide were inserted into the eukaryotic expression vector pTriEx-3 Neo. The constructed chimeric DNA molecules were expressed by transfected CHO cells and the ability of the engineered proteins to interact with FcγRI-expressing cells was confirmed by flow cytometry. The fusion protein induced a strong signal transduction on human monocytes via FcγRI. The expression vector pTriEx-3 Neo containing the described construct was used as a naked DNA vaccine and introduced directly to experimentally humanized NOD/SCID mice with or without boosting with the expressed fusion protein. Immunization with the generated DNA chimeric molecules, and prime-boost with the expressed recombinant proteins induced significant levels of anti-influenza IgG antibodies and strong CTL activity against influenza virus-infected cells in humanized animals. Conclusions: Genetically engineered molecules elicit an efficient anti-influenza immune response in the humanized mice.

P.D.03.01.18
T-CELL AND B-CELL MEMORY IMMUNITY AFTER A SINGLE DOSE BIVALENT HPV VACCINATION COMPARED TO TWO- OR THREE DOSE VACCINATED GIRLS TO 6 YEARS POST-VACCINATION

H. Pasman1, T. Schurink-van't Klooster2, M. Meertens1, S. van der Burg3, F. van der Klei4, A. Buismans4; 1RIVM, Bilthoven, Netherlands; 2LUMC, Leiden, Netherlands.

Vaccines consisting of virus-like particles (VLPs) against Human Papillomavirus (HPV) are currently administered in subsequently two- or three injections. However, it has been shown that just one dose of the bivalent HPV16/18 vaccine results in the seroconversion of all women in a 7 years follow-up vaccination study. The seroconversion is associated with a low prevalence of HPV16/-18 infections suggesting that the induced protection after one dose of vaccine may be long lived. Since T- and B-cell responses are important in the immune protection against HPV, we evaluated these responses in subjects vaccinated with different schedules. Blood was cross-sectional collected and PBMCs were isolated from girls vaccinated at 12 years of age according to a one-, two- or three-dose schedule. T cells producing IFN-γ were determined by ELISPOT after stimulation of PBMCs by VLPs for HPV-16, HPV-18, HPV-31 and HPV-45. HPV-type-specific memory B-cell responses were determined by specific ELISPOT assays after polyclonal stimulation of isolated (CD19+) B cells. Even after 6 years following vaccination HPV-type-specific interferon-gamma producing T cells were detectable. However, these numbers are lower compared to two- and three-dose vaccinated individuals. Memory B-cell responses are detected at least 2 years post vaccination against type 16, 18, 31 and 45. This long-term HPV specific T- and B-cell memory, although further studies will evaluate T-cell responses in more detail. This results suggests that the booster vaccination might not be necessary to induce long-lived HPV-specific immunity.

P.D.03.01.19
Identification of candidate Canxiella burneti T-cell epitopes for a novel human Q-fever vaccine

A. Scholzen1, L. Moise2, G. Richard1, P.M. Reeves1, S. Raoju Paul1, T. A. Brauns1, L. A. Baeten2, R. A. Bowen1, R. Bucal1, C. M. Boyle1, W. D. Martin3, A. E. Sluder2, A. Garritzen4, A. S. De Grood4, M. C. Pazynski4; 1Innotts Laboratories, Oss, Netherlands; 2Epivax, Providence, United States; 3Massachusetts General Hospital, Boston, United States; 4Colorado State University, Fort Collins, United States; 5Yale University School of Medicine, New Haven, United States.

Canxiella burnetii (Cb), the causative agent of Q-fever, is a Gram-negative intracellular bacterium transmitted via aerosol. Regulatory approval of the Australian whole-cell vaccine Q-Vax in the US and Europe is delayed by reactogenicity in previously exposed individuals. The aim of this study was to identify and rationally select CB epitopes for a safe, effective and less reactogenic T-cell targeted human Q-fever vaccine. Immunoinformatic methods were used to predict 65 HLA class I and 50 class II Cb epitopes. HLA binding assays confirmed 89% of class I and 75% of class II predictions, and 11 class II epitopes elicited IFN-γ responses following heterologous DNA/peptide prime-boost immunizations of tgHLA-A2 mice. Q-Fever BCG vaccine is highly immunogenic and elicits long-term specific T- and B-cell memory, although further studies will evaluate T-cell responses in more detail. The results suggest that a booster immunization might not be necessary to induce long-lived HPV-specific immunity.

P.D.03.02.20
Improving the vaccine efficacy of recombinant BCG utilizing the major membrane protein-II (MMPII) antigen against tuberculosis

Y. Tsukamoto1, Y. Mordad2, T. Tamura3, T. Mukai4, S. Mitarai5, Y. Maeda1; 1Department of Mycobacteriology, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan; 2Institute of Mycobacteriology and Research Center, National Institute of Infectious Diseases, Tokyo, Japan; 3Department of Mycobacteriology, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan; 4Department of Mycobacteriology, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

Mycobacterium bovis BCG (BSG) has been used as a vaccine against tuberculosis. However, its efficacy against adult pulmonary tuberculosis is limited. To improve the efficacy of BCG vaccination against tuberculosis, we utilized Major Membrane Protein-II (MMPII) antigen (Ag) from M. tuberculosis (MTB). MMPII Ag is highly immunogenic in terms of activating T cells in vitro. We developed a new recombinant BCG vaccine against MTB with MMP-II Ag from MTB, termed as BCG-DHTM. BCG-DHTM has two characteristics; [1] expresses HSP70-MMPII fusion Ag and (2) induces phagosome-lysosome fusion in cells infected with BCG-DHTM due to depletion of UreC gene in host BCG. BCG-DHTM secreted the fusion Ag and efficiently stimulated immunized cells. To improve the vaccine efficacy of BCG-DHTM, we added proteolysis-inducing signal (Pest sequence) on both ends of HSP70-MMPII fusion gene and introduced into ureC gene depleted BCG, and termed this BCG as BCG-PEST. BCG-PEST secreted the PEST-HSP70-MMPII-PEST fusion Ag and more efficiently induced cytokine production from human APCs than control BCG. DCs infected by BCG-PEST effectively activated naive T cells and promoted IFN-γ production. Furthermore, a single inoculation of BCG-PEST more effectively reduced the multiplication of MTB in murine lungs than control BCG. These results suggest that vaccination with BCG-PEST may efficiently control the growth of MTB in human.

P.D.03.02.21
Cellular immune responses to influenza vaccination in a Dutch cohort of healthy individuals

S. Rasendahl Huber1, A. Turkmor1, M. Hendriks2, R. H. Jacob1, R. A. van Baeten1, N. Y. Bot1, A. ten Brink2, W. Luytjes3, J. van Beek3; 1National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands; 2Sanquin Research, Amsterdam, Netherlands.

Introduction: Influenza virus-specific T cells are limited to induce influenza virus infection and reduce clinical symptoms. However, the induction of T cells by current influenza vaccines remains under debate. Materials and Methods: We performed a vaccine trial in adults and collected PBMCs before, after vaccination, and after 2nd and 3rd vaccinations in two consecutive seasons (2009 – 2011). We analyzed the T cell responses by IFN-γ ELISPOT. PBMCs were stimulated for 18 hours with live influenza A virus or the vaccine components HA or NA. The data was analyzed in a mixed effects negative binomial regression statistical model to correct for pre-existing immunity, sex and age. In depth analyses was performed by Fluorospot and flow cytometry for CD40 in combination with different cytokines. Results: We showed that a single dose of unadjuvanted or adjuvanted vaccine resulted in a significant induction of the response. Interestingly, the revaccination with an unadjuvanted vaccine 1 year after a adjuvanted vaccination induced a significant additional increase in cellular levels compared to the post-vaccinations levels, whereas, a 2nd vaccination, three weeks after the first dose, did not result in a significant increase. In depth analysis by flow cytometry and Fluorospot using the vaccine antigens HA and NA confirmed vaccine specificity, activation status and Th1 cytokine profile of the T cell response. Conclusion: We show the induction of T cells by both adjuvanted and unadjuvanted subunit influenza vaccination. Research was funded by the Dutch government.
P.D3.03.22
The CAMP CHO reporter cell line to replace the in vivo safety test for acellular pertussis vaccines

Pertussis toxin (PTx) is one of the major virulence proteins of Bordetella pertussis. Since pertussis toxin (PTd), the detoxified form of PTx, contributes to protection, this toxin is the key component of all acellular pertussis vaccines. To examine possible toxin content in vaccine batches, each batch is subjected to the in vivo Histamine Sensitization test (HIST). In the last decades the intrinsic limitations of this test - including a lack of mechanistic understanding and animal welfare concerns - have pushed the search for alternative methods. A promising alternative method is based on PTx-induced clustered growth of CHO cells, though the subjective read-out and the limited capacity to discriminate between levels of clustering hamper quantitative detection of PTx levels. On a cellular level, PTx primarily interferes with intracellular pathways that involve CAMP. Based on this phenomenon, we generated a CHO reporter cell line that stably expresses a reporter construct responsive to changes in intracellular CAMP levels. This cell line enables the detection of PTx in a concentration-dependent manner up to a concentration well below the levels detected with the in vivo HIST. More importantly, the cell line detects PTx in the context of an aluminium phosphate adjuvanted aP multivalent vaccine, with a sensitivity equal to the HIST. These results demonstrate that the CHO reporter cell line enables simple, quantitative and concentration-dependent detection of PTx. The cell line therefore offers a promising in vitro method to replace the suboptimal in vivo HIST and in vitro CHO clustering tests.

P.D3.03.23
Chitosan mediated co-delivery of SN38 and Snail-specific siRNA as a useful anticancer approach against prostate cancer
V. Younesi, A. Afkhami, L. Aghabali-Malekzadeh, H. Siahmansouri, M. Ahmadi, M. Yousefi;
1Tabriz University Medical Of Sciences, Tabriz, Iran, Islamic Republic of; 2Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, Tabriz, Iran, Islamic Republic of; 3Department of Immunology, Tabriz University Medical Of Sciences, Tabriz, Iran, Islamic Republic of; 4Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, Tabriz, Iran, Islamic Republic of; 5Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Tabriz, Iran, Islamic Republic of.

Background: Prostate cancer is known as the most common malignancy in men. Chitosan has generated great interest as a useful biopolymer for the encapsulation of small interfering RNA (siRNA). Due to cationic nature, chitosan is able to efficiently encapsulate siRNAs molecules and form nanoparticles. Furthermore, the biocompatible and biodegradable attributes of chitosan have paved the way for its potential application in the in vivo delivery of therapeutic siRNAs. In this study, we aimed to design chitosan/CMD mediated nanoparticles for the efficient encapsulation of the anti-cancer drugs SN38 and Snail-specific siRNA. Methods: Physicochemical characteristics of the synthesized chitosan nanoparticles were analyzed using Scanning Electron Microscopy. Down regulation of targeted genes were confirmed using real time PCR assays. Growth inhibitory properties of the Dual delivery of SN38-Snail siRNA CMD-chitosan nanoparticles were investigated by MTT assay in mouse prostate cancer cells. Results: Our findings revealed that in CMD-SN38-Snail siRNA treated cells the mRNA level of snail decreased from 1.00 to 0.30 (±0.14) and 0.09 (±0.04) after 24 h and 48 h, respectively. Moreover, co-delivery of SN38 and Snail specific siRNA in an appropriate nanocarrier (chitosan nanoparticles) significantly reduced the viability and proliferation rate of the PC-3 cells. Conclusion: In conclusion, CHNs encapsulating SN38 and Snail specific siRNA may represent huge Potential as an effective anti-cancer drug delivery system for the treatment of metastatic prostate cancer.

P.D3.04.01
Transcriptome profiling in blood before and after hepatitis B vaccination shows significant differences in gene expression between responders and non-responders
E. Bartholomeus, N. De Nester1, P. Meyssen1, A. Sue1, N. Keesmaekers1, G. Elia1, H. Janssen, N. Hens1, E. Smith1, V. Van Tendeloo2, P. Beutels2, P. Vandamme1, B. Ogunjimi1,2, K. Laukens1, G. Mortier1,2;
1Center of Medical Genetics, University of Antwerp, Edegem, Belgium, 2Department of Internal Medicine, Faculty of Medicine, University of Antwerp, Edegem, Belgium.

Introduction: As the hepatitis B virus is widely spread and responsible for considerable morbidity and mortality, WHO recommends vaccination from infancy to reduce acute infection and chronic carriers. However, current subunit vaccines are not 100% efficacious and leave 5-10% of recipients unprotected. The development of an effective, safe and deployable malaria vaccine remains an urgent priority for improving global public health. Targeted delivery of antigen to antigen vaccination.

P.D3.04.02
An APC-targeted PRF5S-containing DNA vaccine induce protective immune responses against Plasmodium falciparum
L. Bjerkreim, R. Braathen1, G. Ram Visweswaran1, G. Grødeland1, A. Gudjonsson, G. Labbe1, S. Draper1, B. Bogen1;
1Institute of Clinical Medicine, Oslo, Norway; 2Innen Institute, Oxford, United Kingdom.

The development of an effective, safe and deployable malaria vaccine remains an urgent priority for improving global public health. Targeted delivery of antigen to antigen presenting cells (APC) is an efficient way to increase specific immune responses. Here, we present a DNA vaccine that targets Plasmodium falciparum RBS (PRF5S) antigen to major histocompatibility complex (MHC) class II molecules expressing APC. The PRF5S antigen was cloned into inducible homologous Ig-based molecules (vaccibodies) bearing anti MHC class II sFv. We showed that this vaccine strategy induced high titers of PRF5S-specific antibodies in BALB/c mice that efficiently inhibited the growth of the Plasmodium falciparum 3D7 clone in vitro. Furthermore, the APC-targeted PRF5S vaccine efficiently induced rapid peptide specific IFN-γ T cell responses in mice. To prepare for translation into human vaccination, we constructed a DNA vaccine that targeted HLA class II (HLA-II) molecules which cross-react with MHC class II molecules in several species of larger mammals, including humans. We demonstrated induction of PRF5S-specific antibody responses in vaccinated pigs and that this APC targeted DNA vaccine showed no toxicity. In conclusion, these results reveal a novel vaccination strategy for development of future vaccines against malaria.

P.D3.04.03
Cross-reactivity of anti-dengue human monoclonal antibodies with zika virus (ZIKV)
K. Boonah1, S. Benjamthummarak2, P. Pitsakajul1, P. Ramasoota1;
1Center of Excellence for Antibody Research (CEAR), Faculty of Tropical Medicine, Mahidol University, BANGKOK, Thailand, 2Department of Social and Environmental Medicine, Faculty of Public Health Medicine, Mahidol University, Bangkok, Thailand.

ZIKV is a mosquito-borne disease belonging to the Flaviviridae family with Dengue virus (DENV). ZIKV and DENV serotype 2 share 54% sequence identity of full envelope protein and 100% identity of fusion loop protein. The correlation and homology of ZIKV and DENV may play a role in the pre-existing immunity to DENV. Eventhough, anti-fusion loop antibodies was considered as the major populations found in human immunity, and showed protective activity, the information of antibodies that found in ZIKV infected patients is still limited.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
POSTER PRESENTATIONS

We firstly screened the binding of our 20 human monocular antibodies (huMabs) that showed strong neutralizing activity against all 4 DENV serotypes with ZIKV by IFA. These huMabs characterized their binding regions on domain II of 6 protein and showed cross-reactivity with other flaviviruses like Japanese encephalitis virus (JEV) and some showed strong neutralizing activity against JEV. It was shown that 19 of 20 huMabs showed strong binding activity with ZIKV, as expected by their highly conserved of fusion loop region among the two species. Cross-reactive anti-DENV huMabs with ZIKV were tested for neutralizing activity using focus reduction neutralization test in Vero cell. It was shown that most of huMabs that targeted to envelop DI showed low neutralizing activity. Different with DENV, it was hypothesized that our anti-fusion loop huMabs might not be the target epitope. In a recent study, neutralization and protective activity in ZIKV infected patients. Further study of antibody inhibition ELISA using ZIKV infected serum with several kind antibodies specific to ZIKV spike epitope might showed the real situation of the antibody response of ZIKV infection.

P.D3.04.04
Measles vaccination before 9 months of age results in reduced antibody functionality long-term
I. Brinkman1, A. Butler, J. de Witt, G. Smits, H. N. Hulscher, R. Jongerius, F. van der Klei, N. Rats, D. van Baarle, G. Alter, R. van Binnendijk1;
1National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, 2Ragon Institute of MGH, MIT and Harvard, Cambridge, United States.

Background Measles is one of the most infectious viruses, but effective vaccination significantly reduces the amount of measles cases. However, as maternal antibodies are no longer present around 6 months of age, young infants are at risk until they receive their primary vaccination between 12-15 months of age. This provides a strong incentive to reduce the age of measles vaccination, but the long-term immunological consequences are largely unknown.

Methods Children who received first measles vaccination between 6-8 months or 9-12 months and a second dose at 14 months of age were compared with a control group who only received one dose at 14 months of age. Effectiveness of measles-specific antibodies was determined at 14 months and 1 and 3 years later by systems serology.

Results The majority of children that received a first measles vaccination between 6-12 months induced a significant measles-specific antibody response. However, when vaccinated before 9 months of age, the measles-neutralizing antibody concentrations were lower, and induction of phagocytosis by monocytes and neutrophils as well as complement deposition were affected compared with children who received a first dose after 9 months of age. The reduced effectiveness of antibodies increased over time after vaccination.

Conclusions Early measles vaccination provides short-term protection in the majority of children, but children vaccinated before 9 months of age have a less functional antibodies compared with children vaccinated at a later age. Eventually, this may result in an increasing number of children susceptible to measles long-term.

P.D3.04.05
Transcriptomic analysis of the blood immune response to the rVSV-ZEBOV Ebola vaccine
1University of Siena, Siena, Italy, 2Microbiotec srl, Siena, Italy, 3Leiden University Medical Center, Leiden, Netherlands, 4University of Geneva, Geneva, Switzerland, 5University of Gothaer, Gothaer, Sweden, 6Croat BioPharma LLC, Devens, United States.

rVSV-ZEBOV is a live-attenuated recombinant vesicular stomatitis virus vaccine expressing Ebolavirus glycoprotein G and is the only Ebola vaccine with demonstrated clinical efficacy. Here we studied the blood transcriptomic response in a single dose of vaccine. Whole blood RNA from 64 healthy volunteers, 51 injected either with 10^7 or 5x10^6 PFU of ZEBOV and 13 with placebo, collected at different time points after vaccination, was analyzed by targeted transcriptome sequencing. At each time point, differentially expressed genes (DEGs) were identified with edgeR, ranked by FDR, and used to find biological signatures assessing the activation of 346 blood transcription modules. Between baseline and day 1 after vaccination, 5,469 DEGs were detected. This number decreased over time: at day 35 only 10 DEGs were detected. Functional analysis identified 135 different modules affected by vaccination. Innate immunity pathways were activated from day 1 to day 14. At days 2 and 3, neutrophil modules were downregulated and complement-related modules upregulated. T-cell and cell-cycle associated modules were upregulated at days 7 and 14, while at day 28 no modules remained activated. Correlation analyses of gene expression with ZEBOV glycoprotein-specific antibody titers identified 15 strongly correlated genes at day 14 after vaccination (absolute Spearman’s Rho>0.5, p<0.001).Vaccination with rVSV-ZEBOV induced a strong and durable modulation of innate response associated genes. An algorithm correlating with antibody titers one year after vaccination was developed based on the expression levels of 15 genes. Study supported by IMI-2U Ebola+ program under VSV-EBOVAC [grant 115842] and VSV-EBOPLUS [grant 116068] projects.

P.D3.04.06
Potential impact of maternal vaccination on maternal-child transmission of ZIKV
N. M. Scheltema1, X. M. Kavelaars2, K. Thorburn3, M. P. Hennius4, B. J. van Woensel5, I. A. van der Ent1, J. A. Borghans1, L. L. Bont1, J. D. Yilewicz1;
1University Medical Center Utrecht, Department of Paediatric Infectious diseases and immunology, Utrecht, Netherlands, 2University Medical Center Utrecht, Laboratory of Translational Immunology, Utrecht, Netherlands, 3Department of Paediatric Intensive Care, Alder Hey Children’s Hospital, Liverpool, United Kingdom, 4University Medical Center Utrecht, Department of Paediatric Intensive Care, Utrecht, Netherlands, 5Academic Medical Centre Amsterdam, Department of Paediatric Intensive Care, Amsterdam, Netherlands.

ZIKV, a mosquito-borne flavivirus, is a threat to Mother Child health. Here we studied the blood transcriptomic response of infected women vaccinated with 10^7 or 5x10^6 PFU of rVSV-ZEBOV and 13 with placebo, collected at different time points after vaccination, was analyzed by targeted transcriptome sequencing. At each time point, differentially expressed genes (DEGs) were identified with edgeR, ranked by FDR, and used to find biological signatures assessing the activation of 346 blood transcription modules. Between baseline and day 1 after vaccination, 5,469 DEGs were detected. This number decreased over time: at day 35 only 10 DEGs were detected. Functional analysis identified 135 different modules affected by vaccination. Innate immunity pathways were activated from day 1 to day 14. At days 2 and 3, neutrophil modules were downregulated and complement-related modules upregulated. T-cell and cell-cycle associated modules were upregulated at days 7 and 14, while at day 28 no modules remained activated. Correlation analyses of gene expression with ZEBOV glycoprotein-specific antibody titers identified 15 strongly correlated genes at day 14 after vaccination (absolute Spearman’s Rho>0.5, p<0.001).Vaccination with rVSV-ZEBOV induced a strong and durable modulation of innate response associated genes. An algorithm correlating with antibody titers one year after vaccination was developed based on the expression levels of 15 genes. Study supported by IMI-2U Ebola+ program under VSV-EBOVAC [grant 115842] and VSV-EBOPLUS [grant 116068] projects.

P.D3.04.07
High-dimensional profiling of early immune events following acellular pertussis booster vaccination
J. Gilliard1, P. Braud1, N. Atlasy1, M. Soffritti1, M. I. de Jong1, E. Simonetti1, E. M. Jansen-Megens2, C. Teodossio1, R. de Groot1, G. Pantaleo1, G. Berbers1, C. Fenwick1, H. Stunnenberg1, D. Cliffapoluto1,2;
1Section Pediatric Infectious Diseases, Laboratory of Medical Immunology, Radboud Institute for Molecular Life sciences, Nijmegen, Netherlands, 2Radboud University for Infectious Diseases, Radboudumc, Nijmegen, Netherlands, 3Department of Molecular Biology, Radboud University, Faculty of Science, Nijmegen, Netherlands, 4Swiss Vaccine Institute, Lausanne, Switzerland, 5Leiden University Medical Center, Department of immunohematology and blood transfusion, Leiden, Netherlands, 6National Institute for Public Health and Environment (RIVM), Center for Infectious Disease Control (Cib), Bilthoven, Netherlands.

Many countries continue to experience pertussis epidemics in spite of widespread vaccination. Moreover, increasing disease incidence has been observed in completely vaccinated populations. It is thought that a cohort of 211 children given during infancy programs long-term immunity to pertussis, with waning immunity before adolescence. Whole cell pertussis (wP) vaccines inducing distinct immune profiles. Thus the objective of this study is to apply systems vaccinology to study the early innate immune response to wP booster vaccination in young adolescents primed with either wP or aP vaccines during infancy. We characterized early immune events before, and 24 hours after booster vaccination using complementary tools. Deep phenotyping of circulating immune cells was performed with a specialized mass cytometry (CyTOF) panel for innate responses. In parallel, flow cytometry was used to further characterize the immune response and to obtain single in vivo used to further by flow index sorting, thereby bridging our cytometry dataset and downstream gene expression analysis through single-cell RNA sequencing (scRNAseq). We found that both cytometry datasets display high concordance, including shifts in granulocyte and myeloid populations post-vaccination. scRNAseq and correlation analysis of early innate immunity with long-term pertussis-specific immunity is ongoing. This study provides novel insights into the molecular mechanisms underlying the immune response to aP booster vaccination and provides an important framework for the development of new pertussis booster vaccines.

P.D3.04.08
MHC class II targeted DNA vaccine is most efficient in the induction of protective antibodies against influenza
D. M. Hinke, H. C. Spång, E. Fossum, G. Grødeland, B. Bogen, R. Braathen1, K.G. Jebsen Centre for Influenza Vaccine Research, Oslo, Norway.

Most successful vaccines owe their efficacy to induction of protective antibodies. Vaccine formats that induce high and long-lasting antibody responses will be highly interesting. One approach, APC-targeted DNA vaccination, exercises that transferred cells secrete fusion proteins with targeting units specific for surface molecules on APC. This targeting of antigen to the APC increases delivery of antigen, resulting in improved immune responses. We have benchmarked several different targeting units in their ability to influence the magnitude of the early responses against hemagglutinin (HA) from influenza A.

466
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
We created DNA plasmid vaccines that encode bivalent homodimeric Ig-based molecules (vaccibodies) which express two HA antigens fused to two APC targeting units. The results showed that targeting of KC1-Inh type 1 conventional dendritic cells, using KC1, MP-1a, aCD40 and aCD205 targeting units, preferentially induced IgG2a responses. Simultaneous targeting of several dendritic cell subtypes in addition induced IgG1 responses, as shown for aCD11c, aMHCII, FR-3L and FIC targeting units. IgG1 responses occurred early after immunization but declined relatively rapid over time. IgG2a responses appeared later but lasted longer (>252 days). The overall antibody induction in BALB/c mice depended on the targeting units in the following order: aMHCII+aCD11c>aCD40+Xcl1-MIP-1α=FcγRI-GM-CSF-FR-3L+aCD205. MHC class II targeted DNA vaccines elicited complete short- and long-term protection against influenza virus. Other antigens and T cell assays will be included to confirm the roles of the various targeting units in the magnitude of the responses.

In conclusion, targeting a wide range of APC with MHC class II targeting unit induces protective antibodies against influenza.

P.D3.04.09

Uncovering the induction of varying adaptive immune responses by different Mycobacterium tuberculosis lineages

C. Magalhães1, J. Comas2, M. Saravia3, N. S. Ostorio1

1Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, 2ICVS/3B – PT Government Associate Laboratory, Braga, Portugal, 3ICIBER en Epidemiología y Salud Pública, Valencia, Spain, 4Instituto de Biomedicina, IBV-CSIC, Valencia, Spain, 5IIS - Instituto de Investigación e Innovación en Salud, Porto, Portugal, 6ICIMCB – Instituto de Ciencias e Imunopatologia da Complexidade e Biologia Molecular da Ictericia Bovina, Universidade do Porto, Porto, Portugal.

Tuberculosis (TB) is the deadliest infectious disease in the history of humankind and remains vastly uncontrolled. Active TB results from infection with distinct genetic lineages of the Mycobacterium tuberculosis complex. Interestingly, a strong geographic association between TB cases and specific lineages exists, which is disrupted in the context of HIV-1 co-infection. This fact highlights the relevance of CD4+ T cell-driven immune responses in the interaction between different human/pathogen populations and TB outcome. M. tuberculosis lineage-specific epitope diversity might thus alter the type and level of CD4+ T cell responses generated during infection. Despite the importance of this topic, host-pathogen molecular characteristics influencing immune synapse in TB are not sufficiently studied.

We have developed a genome-wide immunoinformatics approach to identify T cell epitopes that are influenced by the presence of M. tuberculosis lineage-specific polymorphisms. Importantly, it was possible to find significant associations between HLA-binding predictions for a given lineage and the frequency of the HLAs in the human populations with more TB caused by that lineage. Some epitopes were also inferred to have been selected over time by distinct computational molecular evolution methodologies. Overall, this study suggests that specific M. tuberculosis lineage-restricted polymorphisms have been fixed during parallel evolution with the host due to CD4+ T cell pressure. The identification and extensive characterization of varying M. tuberculosis epitopes might be of great relevance for the development of more effective TB vaccination and diagnostics strategies.

P.D3.04.10

Immunisation with a conserved rhinovirus capsid protein induces antibodies that bind a variable neutralising epitope

S. Narean1, C. Numr1, N. Glavilve1, M. Johnson1, G. McLean2

1Cellular and Molecular Immunology Research Centre, London, United Kingdom, 2National Heart and Lung Institute, Imperial College London, London, London, United Kingdom.

Introduction: Human rhinovirus (RV) infections are the principle cause of common colds and precipitate asthma and chronic obstructive pulmonary disease exacerbations. Currently there is no vaccine for RV which is largely due to the existence of ~150 serotypes/strains. We demonstrated previously that immunising mice with highly conserved VP4 and VP2 regions of the RV polyprotein (RV16 VP0) generated broadly cross-reactive protective immunity to RV in vivo. This study investigated and mapped the epitopes of RV16 VP0 that are targets for neutralising polyclonal antibody responses. Materials and Methods: Serum samples from VP0 immunisation and RV challenge studies in mice were used to determine IgG recognition sites by EUSA and in vitro RV neutralisation assay. Peptide pools and individual peptides spanning the immunogen sequence (RV16 VP0) were assessed for IgG binding sites to identify neutralising epitopes. Results: Eight peptide pools containing 15-mer peptides spanning the RV16 VP0 sequence were assessed for binding by antisera obtained from RV16 VP0 immunised and RV challenged mice. We found that peptide pools covering the C-terminus of VP4, N-terminus of VP2 and the neutralising NIm-II loop within VP2 were bound by serum IgG but not by serum IgA. The NIm-II loop peptide pool blocked IgG binding to the immunogen RV16 VP0 but was unable to inhibit IgG neutralisation of RV

P.D3.04.11

Searching for novel vaccine candidates against Echinococcus granulosus combining proteomic and bioinformatic explorations of tegumental antigens

S. Miles1, M. Portela2, M. Cyrklaff2, M. Ancarola1, F. Frischknecht1, R. Durán1, S. Dematteis1, G. Mouriglia-Ettlin1

1Immunology Lab - DEPBIO - UdelaR, Montevideo, Uruguay, 2Institut Pasteur de Montevideo, Montevideo, Uruguay, 3Institute Pasteur de Montevideo, Montevideo, Uruguay, 4Institute for Infectious Diseases, Heidelberg University, Heidelberg, Germany, 5Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPM, UBA-CONICET), Facultad de Medicina, Buenos Aires, Argentina.

Echinococcus granulosus is the helminth parasite responsible for cystic echinococcosis (CE), an important worldwide-distributed zoonotic disease. Development of new vaccines against CE might great have health and economic benefits. Here, we described an innovative vaccine design scheme starting from a tegumental antigens-enriched fraction derived from protoscoleces-named PSEx- already known to induce protection against CE. First, we characterized by mass spectrometry its protein composition. Then, by means of Gene Ontology analyses, we studied the potential biological processes, molecular functions and cellular localizations associated with identified PSEx components. After that, antigenicity predictions and determination of conservancy degree against other organisms were determined. Thus, 10 proteins -identified here for the first time- were proposed as novel vaccine candidates. Furthermore, linear B-cell epitopes free of post-translational modifications were predicted in the whole PSEx proteome through co-localization of in silico predicted and experimentally identified linear B-cell epitopes. Recalling peptides were tested peptide fragments linear B-cell epitopes”, and through BLASTp scanning against all non-helminth proteins, those with 100% identity against any other protein were discarded. Then, secondary structure was predicted for the peptides -free and within their parental proteins- and those showing highly similar secondary structure in both cases were selected. Potentially toxic and/or allergenic peptides were discarded. Finally, selected clean linear B-cell epitopes were mapped within their corresponding 3D-modelled parental protein to assess their possible antibody accessibility, resulting in 14 putative peptide vaccine candidates. At the end, we proposed 10 novel proteins and 14 peptides that deserve further testing as vaccine candidates against CE.

P.D3.04.12

Characterization of the porcine MHC I in the Goettingen minipig

B. von Silva-Tarouca1, M. Wu, D. Zehn1

1School of Life Sciences Weihenstephan, Freising, Germany.

Mice are widely used as an experimental system to explore all aspects of immunity. However, mice and humans have fundamental differences, as demonstrated by the many cases of unsuccessful translation of results from mice to human. Given the strong need to better evaluate therapeutic interventions that are based on biological substances, there is a high demand for well-characterized and robust alternative testing systems. Mice are widely used as an experimental system to explore all aspects of immunity. However, mice and humans have fundamental differences, as demonstrated by the many cases of unsuccessful translation of results from mice to human. Given the strong need to better evaluate therapeutic interventions that are based on biological substances, there is a high demand for well-characterized and robust alternative testing systems. However, as described for an innovative vaccine design scheme starting from a tegumental antigens-enriched fraction derived from protoscoleces-named PSEx- already known to induce protection against CE. First, we characterized by mass spectrometry its protein composition. Then, by means of Gene Ontology analyses, we studied the potential biological processes, molecular functions and cellular localizations associated with identified PSEx components. After that, antigenicity predictions and determination of conservancy degree against other organisms were determined. Thus, 10 proteins -identified here for the first time- were proposed as novel vaccine candidates. Furthermore, linear B-cell epitopes free of post-translational modifications were predicted in the whole PSEx proteome through co-localization of in silico predicted and experimentally identified linear B-cell epitopes. Recalling peptides were tested peptide fragments linear B-cell epitopes”, and through BLASTp scanning against all non-helminth proteins, those with 100% identity against any other protein were discarded. Then, secondary structure was predicted for the peptides -free and within their parental proteins- and those showing highly similar secondary structure in both cases were selected. Potentially toxic and/or allergenic peptides were discarded. Finally, selected clean linear B-cell epitopes were mapped within their corresponding 3D-modelled parental protein to assess their possible antibody accessibility, resulting in 14 putative peptide vaccine candidates. At the end, we proposed 10 novel proteins and 14 peptides that deserve further testing as vaccine candidates against CE.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Abstracts of the 5 POSTER PRESENTATIONS

F. Necchi1, R. Alfieri1, L. Laszló1, D. Rossi1, A. Negrea1, S. Clare1, P. Mastroeni1, A. Saul1, C. A. MacLennan1, S. Rodini1, F. Miccoli1.

1GSK Vaccines Institute for Global Health, Slough, United Kingdom.
2Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom.
3Institute of Membrane Biology, University of Oxford, Oxford, United Kingdom.

Invasive nontyphoidal Salmonella (iNTS) disease is a leading cause of death and morbidity in Africa. The most common pathogens are Salmonella enterica serovars Typhimurium and Enteritidis. The O-antigen portion of their lipopolysaccharide is a target of protective immunity, but no vaccines are currently available, or in clinical trials. Here we investigate the use of Generalized Modules for Membrane Antigens (GMMA) as delivery system for S. Typhimurium and S. Enteritidis O-antigens, and compare this technology to the more traditional conjugate vaccine approach. GMMA are outer membrane vesicles released from genetically engineered Gram-negative bacteria, carrying deletion of the tolR gene or analogue to enhance their production. Salmonella tolR strains were generated for GMMA production. S. typhimurium and S. enteritidis O-antigens were extracted from corresponding wild-type bacteria and conjugated to CRM197. Purified GMMA and conjugates were characterized, showing similar O-antigen structural characteristics. When compared in mice, GMMA gave higher anti-O-antigen IgG titers compared to conjugate, in the absence of Alhydrogel. With Alhydrogel, antibody levels were similar. Antibody isotype profile was also investigated, showing a diverse Ig subclass repertoire induced by GMMA, with greater serum bactericidal activity compared to that induced by the conjugate vaccine. In an in vivo mouse infection model, bacterial colonisation was significantly reduced with GMMA, with S. Typhimurium counts lower with GMMA and with S. Enteritidis burden similar with both vaccines. Overall, simplicity of manufacturing process and low cost of production, coupled with encouraging immunogenicity data, make GMMA an attractive strategy for the development of a nontyphoidal Salmonella vaccine compared with established conjugate vaccine technology.

P. Mastroeni1
1Laboratory of Mucosal Entry of HIV-1 and Mucosal Immunity, Department of Infection, Immunity and Inflammation, Cochin Institute, CNRS UMR 8104, Paris, France, 2Institute of Membrane Biology, University of Oxford, Oxford, United Kingdom.

Pertussis remains an important cause of infant mortality despite global infant vaccination programs. The recent resurgence of Bordetella pertussis infections worldwide is caused by new vaccines inducing longer-lasting protection. The magnitude and persistence of pertussis-specific immunity was explored during a clinical trial (ClinicalTrials.gov NCT01529645) evaluating the safety and immunogenicity of different doses of booster vaccines of acellular pertussis (aP) in combination with diphtheria and tetanus antigens (Tdap) in adults. Participants received Tdap formulations, containing either the genetically or chemically detoxified pertussis toxin (PT) in combination with the filamentous hemagglutinin (FHA) and pertactin (PRN) antigens. Frequencies of antigen-specific plasmablasts (pPBL) at day 0 and day 8 after vaccination, CD4+ T cells (at baseline, DB and D30 after vaccination) and Memory B Cells (MBC) (at baseline, on D30 and D365 after vaccination) were assessed. All vaccine formulations expanded antigen-specific IgG MBC and PB against FHA, PRN and PT. Remarkably, genetically detoxified PT induced higher frequencies of MBC than chemically detoxified PT, that remained higher one year after vaccination. Lack of IgM and IgG of low frequencies of IgM MBC in any of the vaccination groups indicate pre-existing immunity to pertussis antigens. Frequencies of PRN, FHA and PT-specific CD4+ T cells 1 month post-vaccination were similar. Overall, the major results were that the evidence of a stronger propensity of genetically detoxified PT to induce immunological responses which might prove promising for next generation pertussis vaccines.

F. Schiavetti1, E. Faenz1, K. Burichi1, E. Borgagn1, M. Bardelli1, F. Spennieri1, A. Seubert1, M. Piazza1, G. Leroux-Roels2, S. Berthelet1, O. Finca1, G. Del Giudice1.

1GSK, Siena, Italy, 2Centre for Vaccinology, Ghent University and University Hospital, Ghent, Belgium.

Comparison of incidence of rare adverse reactions resulting from vaccination against serotypes A, B and C of serogroup B meningococci highlights the importance of such events as a novel and essential component of niches for IgG-secreting plasma cells in the BM, and furthermore qualifies as a unique therapeutic option to ablate selectively IgG-secreting plasma cells. We here report that the protein SiiE of Salmonella typhimurium is required and sufficient to prevent an efficient humoral immune response, selectively reducing the numbers of IgG-producing plasma cells in the BM. Interestingly, SiiE is currently available, albeit multiple drug-resistant non-typhoidal Salmonella is already highly prevalent. We here report that the protein SiiE of S. Typhimurium is required and sufficient to prevent an efficient humoral immune response, selectively reducing the numbers of IgG-secreting plasma cells in the bone marrow (BM). Attenuated SiiE-deficient S. Typhimurium induces high and lasting titers of specific and protective IgG, and qualifies as the first efficient vaccine for the serotype. An SiiE-derived peptide with homology to laminin β1 is sufficient to ablate selectively IgG-secreting plasma cells from the BM, identifying laminin β1 as a novel and essential component of niches for IgG-secreting plasma cells in the BM, and furthermore qualifies as a unique therapeutic option to ablate selectively IgG-secreting plasma cells in autoimmune diseases and multiple myeloma.

P. D.3.04.13

Comparative Immunogenicity protective efficacy of equivalent bivalent Generalized Modules for Membrane Antigens (GMMA) glycoconjugate vaccines against nontyphoidal Salmonella.

D. Tudor1, M. Ducournau1, M. Khassani2, L. Xu3, M. Bomsel4.

1Laboratory of Mucosal Entry of HIV-1 and Mucosal Immunity, Department of Infection, Immunity and Inflammation, Cochin Institute, CNRS UMR 8104, Paris, France, 2INSERM U1016, Paris, France, 3Université Paris Descartes, Sorbonne Paris Cité, Paris, France.

Introduction: Although raising broadly neutralizing antibodies (bNAbs) are the main goal of vaccination against HIV-1, growing evidence show that protecting antibodies could also control viral infection by Fc-mediated antiviral activity, such as antibody dependent cellular cytotoxicity (ADCC). Until now, mainly gp120 and gp41 HIV-1 envelope-specific IgG have been shown to mediate ADCC whereas the ADCC potential of HIV-1 envelope-specific IgA remains elusive, despite the prevalence of IgA at mucosal level, the main portal entry for HIV-1 infection. The capacity of HIV-1 envelope-specific IgA to induce FcαR-mediated ADCC was evaluated by flow cytometry using 2FS-IgA genetically engineered from the broadly neutralizing gp41-specific 2FS-IgG we have previously reported to induce ADCC. Effector cells were primary monocytes and target cells were CD4+ T lymphocytes either infected with the recombinant HIV-1 subtype A and B or C or coated with the P1- A, -B, and -C peptides covering the MP1 region of gp120 from subtype A and B. In vivo results indicate the show here that 2FS-IgA, targeting subtype A and B, but not Gp41, engages FcαRI (CD89), expressed on human monocytes, to induce the lysis of subtype A and B, but not C. HIV-1 infected cells and of P1-A and -B, but not C, coated cells by ADCC. Furthermore, the 2FS-IgA cooperates with 2FS-IgG as well as with the bNAb gp41-specific 10E8-IgG to enhance target-cell lysis by ADCC. Conclusion: These results indicate that inducing IgA by vaccination, especially those targeting gp41, together with IgG could strength HIV-1 mucosal protective immunity against infection by affecting ADCC.
EMCV-infection. Moreover, we obtained evidence for functional diversity in virus-induced EV, by showing that EV subpopulations differed in their efficiency to transmit infection. To investigate which APC types in the blood are directly infected, PBMCs were infected with YF-17D in vitro and viral RNA content was measured in different APC subpopulations by RNA flow cytometry. YF-17D (+) strand RNA was detectable in DCLs and monocytes and the percentage of infected cells increased with inhibition of type I IFN induction pathways.

Methods

Background

Results:

To investigate which APC types in the blood are directly infected, PBMCs were infected with YF-17D in vitro and viral RNA content was measured in different APC subpopulations by RNA flow cytometry. YF-17D (+) strand RNA was detectable in DCLs and monocytes and the percentage of infected cells increased with inhibition of type I IFN induction pathways.

Conclusions

These results suggest that monocytes and DCLs play a major role in the immune response to YF-17D infection. We propose that the success of the YF-17D vaccine is based on controlled viral replication within monocytes and DCLs leading to activation and optimal presentation of viral antigens.

Detection of YF-17D viral RNA in human immune-reacting fluid cytometry

Host cells respond to encephalomyocarditis virus infection by releasing diverse populations of extracellular vesicles

Shingles vaccine works yet why are uptake rates declining in the UK?

The role of extracellular vesicles (EV) in modulating the immune response to viral infection is increasingly recognized. Recently, it was demonstrated that naked viruses can be applied to functionally separate and quantify EV subsets. In addition, protein content and infectivity of EV were analyzed by western blotting and end-point titration.

EV released by EMCV-infected cells were separated into subpopulations by differential ultracentrifugation and density gradient purification. High-resolution flow cytometry was used to functionally separate and quantify EV subsets. In addition, protein content and infectivity of EV were analyzed by western blotting and end-point titration.

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Abstracts of the 5th European Congress of Immunology - EC1 2018 - Amsterdam, The Netherlands

P.D4.01.02

Preventing type I IFN production by the aminopeptidase IRAP in neonatal alveolar macrophages in response to RSV

D. descamps1, C. Droja2, D. Loubertet1, L. Saveranu1, J. Schwartz3, S. Riffault1

1INRA, Jouy-en-Josas, France, 2INSERM, Paris, France.

Introduction: Respiratory Syncytial Virus (RSV) is the major cause of neonatal lower respiratory tract infection. Neonatal mice have an important defect in the pulmonary production of type I interferons (IFN-I) during RSV infection compared to adults. Recently, it has been shown in adult mice that alveolar macrophages (AM) constitute the main source of IFN-I upon RSV infection. The ability of neonatal AM to produce IFN-I in response to RSV infection remains to be determined. IFN-I responses can be triggered following RSV infection of neonatal immune receptors of the cell, whereas their activation and intracellular trafficking are tightly controlled. Thus, Insulin-Responsive Aminopeptidase (IRAP), an amino acid transporter necessary for anchoring the endosomes to the actin network, has been described to participate in regulating of IFN-I production in dendritic cells. Thus, we characterized the ability of neonatal AMs to mobilize IFN-I pathways upon RSV infection and we determined the contribution of IRAP in this response.

Methods: Neonatal or adult AMs from IRAP-deficient (IRAPKO) mice and wild-type (WT) were isolated and exposed ex vivo to RSV or different ligands of innate receptors to in order to compare IFN-I responses.

Results: RSV infection of adult WT AMs induced the production of IFN-I and the up-regulation of interferon-stimulated gene transcripts, while these responses are very low in neonatal AMs. However, IFN-I responses were significantly increased in neonatal IRAPKO AMs following RSV infection.

Conclusion: These data suggest that IRAP plays a key role in regulating responsiveness of AMs to produce IFN-I following RSV infection during the neonatal period.

P.D4.01.03

Evasion of NK cell responses by a cytomegalovirus-encoded soluble CD48 homolog

P. Engel, P. Martinez-Vicente, D. Darre, A. Angulo

University of Barcelona, Barcelona, Spain.

Cytomegaloviruses (CMVs) have developed a wide range of mechanisms to subvert host immunity and establish successful long-term infections. To accomplish it, they encode a large repertoire of immune modulator genes, some of which derive from their host genomes after being captured at different points during host-virus co-evolution. CD48 is a GPI-anchored protein that contains an ectodomain composed by 2 immunoglobulin (Ig) domains. Via its N-terminal Ig domain, CD48 recognizes the cell surface receptor 2B4. Engagement of 2B4 by CD48 results in the regulation of cytotoxic T lymphocyte and NK cell functions. We have recently reported the presence of a number of CD48 homologs (vCD48s) encoded by different CMVs. Here, we have characterized the three vCD48 of owl monkey CD48, showing that they are highly glycosylated transmembrane proteins which display very distinctive structural and biochemical properties. Among them, only A43, the viral CD48 that exhibits the highest amino acid identity with host CD48 is able to bind to 2B4, with the two other vCD48s having diverged to perform 2B4-independent functions. Interestingly, A43 is a soluble protein, released from the cell after being proteolytically processed through its stalk region. Kinetic studies reveal that A43:2B4 interactions are of exceptional affinity and highly stable, resulting in a 1000-fold drastically reduced as compared with that established between CD48 and 2B4. We demonstrate that purified soluble A43 is capable to efficiently abrogate CD48:2B4 interactions. Furthermore, this viral protein severely impairs 2B4-mediated NK cell cytotoxicity. Thus, A43 acts as a functional virally-encoded CD48 decoy receptor.

P.D4.01.04

TNFα3 negatively regulates Cutibacterium acnes-induced inflammatory events in human epidermal keratinocytes

L. Erdei1, B. S. Bolla1, G. Tóth1, E. Urbán1, L. Kemény1, K. Szabó1

1Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary; 2Institute of Clinical Microbiology, University of Szeged, Hungary, Szeged, Hungary, MTA-SZTE Dermatological Research Group, Szeged, Hungary, Szeged, Hungary.

Human epidermal keratinocytes can recognize the skin microbiome, such as Cutibacterium acnes (C. acnes), through TLRs, and induce innate immune and inflammatory events.

Little is known about endogenous regulators which can control these events and protect the host from the prolonged inflammation. For that, we aimed to analyze whether TNFα3, a negative regulator of NF-κB signaling has a role in TLR ligand and C. acnes-induced innate immune and inflammatory events. In our studies we used a human immortalized keratinocyte cell line (HPV-KER). We analyzed TNFα3 expression in response to different TLR ligands and C. acnes, and followed NF-κB activation using a luciferase reporter assay and by monitoring the expression of pro-inflammatory mediators upon TNFα3-silencing by real-time RT-PCR, western blotting and ELISA analysis. Our results show that TNFα3 mRNA and protein expression significantly increased in response to C. acnes. When analyzing the C. acnes-induced signaling events in details, we found that bacterial treatment also induced a significant, transient and dose-dependent upregulation of TNFα3 expression, which were dependent on JNK and NF-κB pathways. Downregulation of TNFα3 levels by siRNA-mediated silencing increased the basal NF-κB promoter activities and the mRNA expression of selected pro-inflammatory cytokines and chemokines, such as TNFα, IL-1α, IL-6, CXCL8 and CCL5. Parallel to that, secreted IL-6, CXCL8 and CCL5 levels also increased. Based on our results, TNFα3 is one of the negative regulators in keratinocytes, which may control P. acnes-induced signaling events and play a role in the maintenance of epidermal homeostasis.

P.D4.01.05

CD8 T CELLS IN EXPERIMENTAL ZIKA VIRUS INFECTION

N. Ghabdan Zanluqui, C. Manganelli Polonio, L. Gomes de Oliveira, C. Longo de Freitas, J. Schatzman Peron

Biomedical Sciences Institute, São Paulo, Brazil.

Introduction. The relevance of Zika virus (ZIKV) infection study was highlighted by the large number of infants born with microcephaly and some adults exhibited cases of Guillain-Barré syndrome caused by ZIKV infection. It is known that ZIKV, like other flaviviruses, has the ability to modulate innate and adaptive immune response of the host. Thus, in this work we aim to evaluate the role of CD8 cells in controlling viral replication and diseases progression in murine infection by ZIKV. Methods. ZIKV (BeH815744 strain) was used to infect CD8-/- mice and WT. Infection was monitored by viremia analysis. ZIKV-infected WT and CD8-/- mice did not show any morbidity or clinical sign in the course of infection. Interestingly, in the peak of infection CD8-/- showed high viremia compared to WT while WT and CD8-/- mice showed similar levels of viremia. To study the immune response to ZIKV, we characterized the CD8+ T cells response specific for ZIKV antigens using pentamers. We measured their frequency, accompanied by CD8+Foxp3+ and CD4+Foxp3+ cells, while a decrease of IL-10 secretion in CD8-/- mice was detected. Conclusion. CD8 T cells could play an important role in controlling viral replication and disease progression in murine infection by ZIKV.

P.D4.01.06

EBV-specific CD8+ T cells are exhausted and senescent in Multiple Sclerosis patients

G. Guerrera1, D. F. Angeli1, S. Raggetti2, F. Garagona3, C. Gasperini1, R. Placido1, G. Borsellino1, L. Battistini2

1Neuroimmunology Unit, Santa Lucia Foundation, Rome, Italy, 2Department of Neuroscience “Lancisi”, San Camillo Hospital, Rome, Italy.

Multiple sclerosis (MS), the most common chronic inflammatory disease of the central nervous system (CNS), is associated with an increased Epstein-Barr virus (EBV) sero-prevalence and high immune reactivity to EBV. While EBV infection alone cannot explain MS development, our hypothesis is that, in susceptible individuals, defects in the control of EBV facilitate the establishment of viral infection and of continuous cycles of inflammation in the CNS, due to the recruitment and activation of inflammatory cells in the brain. To study this, we characterized the immune response to EBV in MS patients expressing and surviving EBV lytic and latent antigens using pentamers. We measured their frequency, activation and functional state in MS patients compared with healthy donors (HD). In MS patients, CD8+ cells specific for EBV antigens show the phenotype of terminally differentiated and functionally impaired and senescent cells, likely due to chronic viral stimulation; secondly, we found that some EBV-specific T cells are exhausted and express PD-1.

P.D4.01.07

y6 T-cells in murine Cytomegalovirus infection

A. M. Hahn1, S. Sell2, A. Schneider3, S. Brey1, A. Donaubauer1, M. Mach1, T. H. Winkler2

1Nikolaus-Fiebig Center for Molecular Medicine, Institute of Genetics, Department of Biology, University Erlangen-Nuremberg (FAU), Erlangen, Germany, 2Institute for Clinical and Molecular Virology, University Hospital Leverkusen, Germany.

Introduction: Upon Cytomegalovirus (CMV) infection immunocompetent patients are at substantial risk for developing severe organ disease eventually leading to multi-organ failure. Existing viral escape mechanisms to anti-viral drugs plus missing approved vaccination currently designate CMV as major health issue. Previous findings showed that y6 T cells can effectively control murine CMV (mCMV): remarkably when conventional immune mechanisms (like CD8, B or Natural Killer cells) are insufficient or absent, corresponding to the immune-suppressed state in transplant recipients and neonates. In accordance with recently published data from the human system, these studies suggest adaptive features of y6 T cells.
However, recognition mechanisms, antigen specificity and the formation of a classical memory remain enigmatic. Material & Methods: To examine, to what extent the T cell response to recombinant TCR (TcR) was in the presence of viral antigens, we monitored 8 TCR repertoires in TCRα+ mice for clonal diversity. In different organs RNA-based immune-profiling of V(D)J rearranged complementary-determining regions (CDR) from selected receptor chains (TRGVL, TRGV4, TRDVL, TRAV1S/DRV6) was performed in a time kinetic manner. Taking advantage of a fluorescent reporter system for TCR activation and hybridoma technology we identified T cells with the expression of mCDV infected target cells in vitro. Results & Conclusions: Alterations in clonality and focusing of the CDR3 length distribution after virus exposure implicate an antigen-driven response with TCR involvement. Ongoing experiments will then define the TCR engagement in more detail via mutational studies and the exploitation of virus deletion mutants. CMV-reactive T cells are a promising target for future cellular vaccines.

P.D4.01.08
Association of autoantibody to rods, rings with hepatitis C virus load

Y. Lakhoua Gorgi1, J. Abdellatif2, M. Jellouli3, M. Majdoubi1, L. Mouelhi1, T. Ben Abdallah1, L. Sfar1, T. Dhaouadi1
1Research Laboratory in Immunology of Renal Transplantation and Immunopathology (LR03SP01), Tunis, Tunisia; 2Department of Gastroenterology Charles Nicolle hospital, Tunis, Tunisia.

INTRODUCTION: Chronic infection with hepatitis C virus (HCV) is an indication for treatment with Ribavirin, a nucleoside analogue of guanosine. The cellular targets of Ribavirin are 2 enzymes (CTPS1 and IMPDH2) which are essential for the CTP and GTP nucleosides’ synthesis. It has been reported that in treated patients, the appearance of rare autoantibodies directed against these 2 enzymes and giving the appearance of Rods and Rings (RnR) is generally correlated with a viral escape to treatment with Ribavirin. MATERIAL AND METHODS: In this context, anti-RnR antibodies (Ab) were detected by IFI on HEp-2 cells in 142 HCV patients under Ribavirin: 74 patients with positive HCV-PCR (G1) and 68HCV-PCR negative patients (G2) matched in age and sex with G1 and served as controls. RESULTS: The frequency of anti-RnR Ab was significantly higher in HCV-PCR positive patients (25.7%) than in those with negative HCV-PCR (6%) and 10- to 15-fold. Furthermore, in G2, the viral load was significantly higher in patients with anti-RnR Ab (6.05378.32 IU/ml vs 3.80527.38 IU/ml), p=3.13 10E-6. Nevertheless, the presence of anti-RnR Ab was not correlated neither to gender nor to age. CONCLUSION: Anti-RnR Ab seems to be associated with increased replication of HCV and would be predictive of viral escape under Ribavirin.

P.D4.01.09
Cyclokinones upon Bovine Respiratory Syncytial Virusand Bovine Viral Diarrhea Virus vaccination in dairy cows

S. Lee, Y. Kim, S. Ryu, A. Prasad
Chonbuk National University, Iksan, Korea, Republic of

Bovine respiratory disease (BRD) is defined as a “disease complex”, which is usually caused by a variety of pathogens including Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza 3 (PI3), and Bovine Viral Diarrhea Virus (BVDV). The key to reduce BRD is to vaccinate against these viruses. Although vaccination is an effective measure in reducing the risk of BRD in cattle, BRD losses remain significant. Increasing the efficacy of vaccination depends on elucidating the protective immune response to different antigens included in vaccines and understanding the cyclokinones responses which reflect complicated immune responses against vaccinated antigens. This study evaluated the serum antibodies present in plasma from 120 healthy rabbits vaccinated with BRSV, BVDV and PI3. Antibody titers and cyclokinones were measured in more than 100 cows at 0, 1, 2, 4, 24 weeks post vaccination. Upon vaccination, the levels of antibodies against viral respiratory pathogens were significantly enhanced in serum and innate cytokines such as TNF-α and IL-6 were also increased at 1 wk. Cyclokinones related with helper T cells including IFN-γ, IL-4 and IL-17 were also modulated by vaccination and correlated with antibody levels in serum. The present study may provide better understanding of immune responses against viral pathogens related with BRD upon vaccination.

P.D4.01.10
Mast cells promote thrombocytopenia during dengue virus infection through the release of serotonin.

M. Maiz, C. Mantri, A. Rathore, A. St. John; Duke-NUS Medical School, Singapore, Singapore.

Introduction: Thrombocytopenia, a reduction in platelet counts, is a classical trait of dengue fever (DF), which is caused by infection by dengue virus (DENV). DENV can activate mast cells (MCs), which are innate immune sentinels. This results in the release of MC mediators that can influence the severity of dengue vascular leakage. We hypothesized that MC-derived products also contribute to thrombocytopenia during DENV infection.

Materials and methods: Wild type (WT) and MC-deficient (Sash) mice were infected with DENV. Blood and spleens were harvested post-infection for analysis of platelet activation and turnover by flow cytometry. WT mice were treated with MC stabilizer, ketotifen, or 5HT receptor antagonist, ketanserin. Sash mice were reconstituted with MCs or administered exogenous serotonin to determine the role of mast cell derived serotonin during DENV infection.

Results: DENV infection of WT mice induced thrombocytopenia, which was absent in Sash mice and reduced in mice treated with ketotifen. Reconstitution of the Sash mice with MCs restored the phenotype of thrombocytopenia. Pharmacological inhibition of serotonin with the 5HT receptor antagonist, ketanserin, in DENV-infected WT mice reduced thrombocytopenia compared to vehicle treated mice. Conversely, treatment of DENV-infected Sash mice with exogenous serotonin restored the thrombocytopenic phenotype that is absent in Sash mice.

Conclusions: Our findings have demonstrated that MC release of serotonin contributes to thrombocytopenia during DENV infection, revealing a potential therapeutic target of disease.

P.D4.01.11
A new in vivo model to study protective immunity to Zika virus infection in mice with intact type I interferon signaling

L. Nazerai1, A. Skak Schiller, P. Overbeck Sharma Rasmussen, S. Buus, A. Stryhn, J. Pravsgaard Christensen, A. Randrup Thamsen; Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark.

To date, recent Zika virus (ZIKV) infection and severe neurologico-pathological complications, microcephaly in the fetus and Guillain-Barre syndrome in adults, underscores the necessity for a protective vaccine. Unlike the situation in humans, ZIKV can only replicate effectively in mice when type I IFN signaling is interrupted. As type I IFN also impacts the adaptive immune response, mice with a defect are not optimal for a comprehensive immunological analysis. In this report, we show that even in wild-type (WT) mice, (intracranial) i.c. infection with low doses of ZIKV causes marked local virus replication and lethal encephalitis in naive mice. Furthermore, peripheral infection of WT mice with low doses of virus induces a significant immune response, which provides long-lasting protection of WT mice from a fatal outcome of subsequent i.c. challenge. Therefore, combining peripheral priming with later i.c. challenge represents a new approach for studying the adaptive immune response to ZIKV in mice with intact type I IFN response. Using a combination of adoptive transfer, antibody-cell based cell depletion, and gene targeting, we show that the key protective factors in type I IFN replete mice is humoral immunity. CBB T cells are not essential in mice with preformed specific antibodies, but under conditions where initial antibody levels are low, effector CBB T cells may play a role as a back-up system. These results have important implications for our understanding of innate immunity to ZIKV infection and for Zika vaccine design.

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P.D4.01.12
Reactivity of the immune system in rabbits experimentally infected with antigenic variants of RHDV (rabbit haemorrhagic disease virus).

P. Niedzwiedzka-Rystew1, W. Tokarz-Deptula2, W. Deptula1
1University of Szczecin, Faculty of Biology, Department of Immunology, Szczecin, Poland; 2University of Szczecin, Faculty of Biology, Department of Microbiology, Szczecin, Poland.

The aim of the study was to evaluate the immunogenicity of 6 selected RHDVα (V97, Tripts, Hartmannsdorf, P97, 9905 and 72V/2003) obtained in 1996-2003, based on selected natural and acquired immunity parameters. Clinical symptoms and mortality were also recorded. The experiment was performed on 120 rabbits of Polish mixed breed rabbits. Blood and serum was collected at hour 0, followed by 4, 8, 12, 24, 36, 48, 52, 56 and 60 h p.i. Analyzing the picture of changes in natural immunity parameters, the RHDVa strains does not confirm the observations with classical RHDV strains, where time of isolation significantly influenced their diversity. However, the time of receiving RHDV strains does not confirm the observations with classical RHDV strains, where time of isolation significantly influenced their diversity.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 471
Mucosal Transforming Growth Factor-β1 (TGF-β1), a pleiotropic, potent immunoregulatory cytokine, demonstrated to manage phosphorylated AKT and IFNγ expressions, are associated with intestinal epithelial cells (IECs) survival in macaque colon explants and suggest a potential role of mucosal TGF-β1 in regulating intestinal homeostasis and IEC integrity. It is important to monitor and further explore the role of mucosal TGF-β1 in HIV/SIV pathogenesis and HIV/SIV enteropathy, which may lead to the development of improved therapeutic strategies to prevent IEC damage and systemic immune activation during acute and chronic infection. Our data showed an increased production of intestinal TGF-β1 in T-, B- and non-T/B cell populations during acute and chronic SIV infection in rhesus macaques, without a change in the expression of TGF-βR1. The increased levels of immunosuppressing TGF-β1 were also associated with increased production of IFNγ, suggest the lack of TGF-β1 mediated anti-inflammatory responses in SIV infection. An appropriate balance between inflammatory and anti-inflammatory cytokine responses are crucial for maintenance of a successful immune responses in HIV infection. TGF-β1 induced immune defects contribute to intestinal inflammation, loss of tight junction protein, and apoptosis by overexpression of SMAD3, and downregulation of inhibitory SMAD7 transcription factors. Together, these results indicate that SMAD mediated pathway play a crucial role in regulating TGF-β1 expression and that was thought to be a key contributor to the dysfunction of CD4+ and CD8+ T-cells, IEC apoptosis and disease progression.

PREPARATION OF IMMUNE RESPONSES TO INFLUENZA INFECTION


1University of Pennsylvania School of Veterinary Medicine, Philadelphia, United States. 2University of Pennsylvania School of Veterinary Medicine, Philadelphia, United States.

Influenza is a leading cause of respiratory mortality and morbidity. While inflammation is necessary for fighting infection, a fine balance of anti-viral defense and host tolerance is crucial to the successful outcome. Circadian rhythms form an anticipatory system wherein various aspects of cellular physiology and behaviors oscillate across a 24h period; these rhythms have been known to modulate the immune responses. However, the role of circadian rhythms in influenza infection is not well known. To elucidate this role, we infected C57/B6 mice with influenza virus (PR8) either at the beginning of their active phase (ZT11) or at the beginning of their rest phase (ZT23). Mice infected at ZT11 had more than fourfold higher mortality, more weight loss and worse clinical scores than mice infected at ZT23. This was the result of exaggerated inflammation as evident in higher bronchoalveolar lavage cell counts (including more neutrophils on day 2 post-infection) and worse lung pathology on day 6. Interestingly, NK cells were present in higher numbers in the ZT23 than in the ZT11 group at the early phase of infection. Further, pathway analyses of our transcriptomic data supported the role of higher inflammation in the ZT11 group. Thus, circadian regulatory networks, make the host at ZT11 mount an inefficient viral clearance response, wherein more inflammation is needed to achieve similar clearance of the pathogen, resulting in more host injury and death. These data suggest that the circadian regulation of host inflammation and tolerance, rather than viral burden determines the outcome of influenza infection.

The evidence of intestinal Tumor Growth Factor-beta1 associated with increased intestinal epithelial cell apoptosis in pathogenic simian immunodeficiency virus infection


The participation rate was 48.77%. The average age of the staff was 45± 2 years. The prevalence of HBsAg in the study population was 13%. HBsAg carriers were mostly middle-aged. The prevalence of HBsAg among hospital staff remains high. Preventive measures such as vaccination of people unprotected from HBV and awareness of the risks associated with practice in the hospital environment are needed.

Regulation of intestinal Tumor Growth Factor-beta1 associated with increased intestinal epithelial cell apoptosis in pathogenic simian immunodeficiency virus infection

P.D4.01.18  
Effect of latent cytomegalovirus (CMV)-infection on the immune response to influenza with age  
S. P. H. van den Berg1, R. J. Jacobi2, M. Hendriks3, R. van Schuijlenburg1, Z. Euler1, S. Rożalska1, P. Żelechowska1, P. D. van der Merwe1, M. Hendriks3, 1National Institute for Public Health and the Environment, Bilthoven, Netherlands, 2University Medical Center Utrecht, Utrecht, Netherlands.

Older adults are at higher risk for influenza-virus infection and for influenza-related death compared to younger adults. Unfortunately, influenza-vaccine responses are impaired in older adults due to ageing of the immune system (immunosenescence). Latent infection with cytomegalovirus (CMV) is generally thought to aggravate this state of immunosenesence. We studied whether CMV-infection impairs the immune response to influenza-virus. The effect of CMV-infection on the influenza-vaccine antibody response has not been investigated before, with very contrasting results. Meta-analysis of these studies revealed a trend towards a negative effect of CMV-seropositivity on the response to influenza vaccination. funnel-plot analysis suggests, however, that this is due to publication bias. Differences between studies might be due to confounding effects of preexisting immunity, which influences immune responses to seasonal influenza vaccines. Therefore, we investigated the influence of CMV-infection on the antibody response to the pandemic influenza virus of 2009, to which pre-existing immunity was negligible. Despite a negative effect of age, no effect of CMV-infection on the influenza-vaccine response was observed. Finally, we investigated the effect of CMV-infection on influenza-virus-specific T-cells in a cohort of influenza-virus-infected elderly. Despite a clear effect of CMV-infection on the phenotype of the total T-cell pool, CMV-infection did not impair the T-cell response to influenza-virus infection. Unexpectedly, the height of the T-cell response to CMV and influenza virus correlated positively, showing that the CMV-host balance is still not fully understood. Altogether, we find no evidence in human for impairment of the immune response to influenza vaccine or infection by CMV.

P.D4.01.19  
Prevalence of broadly neutralizing antibody responses in HIV-1 infected injecting drug users  
Z. Euler1,2, T. van den Kerkhof3, K. Warmink4, S. Rożalska1, P. D. van der Merwe1, M. Hendriks3, P. Żelechowska1, E. Brzezińska-Blaszczyk1, J. S. Friedland;1,2,3,4
1Institute of Medical Virology, Zurich, Switzerland, 2Department of Molecular Cell Mechanisms, Medical University of Lodz, Lodz, Poland, 3Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland, 4Institute of Medical Virology, Zurich, Switzerland.

The understanding factors in the development of broadly neutralizing antibody (bnAb) responses in natural infection can guide vaccine design aiming to elicit protection against HIV-1 acquisition. Most of the studies to identify and study development of bnAb responses were performed in individuals who had become infected via homo- or heterosexual HIV-1 transmission, however the prevalent and characteristics of bnAb responses in injecting drug users (IDUs) remains to be established.

A retrospective cohort study on the prevalence of bnAb responses in HIV-1 infected individuals, who reported injecting drug use as the only risk factor (50 male and 35 female participants of the Amsterdam Cohort Studies) was conducted. The study revealed a significantly lower prevalence of bnAb responses compared to MSM, which was no longer evident when women were excluded from the IDU group.

interestingly, more elite neutralizers were found in the IDUs with 6% of male IDUs being elite neutralizers as compared to only 0.3% amongst MSM and 0% of female IDUs. Gender, transmission route and CD4+ count at set point, were independently associated with bnAb responses but not viral load for the IDUs or HIV-1 envelope glycoprotein sequence diversity within the first year of infection. Similar observations were seen in the Swiss Cohort, indicating that injecting drug use may influence the development of potent humoral immune responses, with a stronger effect in females.

We draw the realization that the emergence of bnAbs may be dependent on multiple factors, not only host or viral but also behaviour.

P.D4.01.20  
Identification of new biomarkers to distinguish between a bacterial or viral infection in children  
J. Zandstra1,2, M. H. Iansen1, S. Zeekerd1, T. W. Kuijpers1,3
1Dept of Immunopathology, Sanquin Blood Supply, Division Research, Amsterdam, Netherlands, 2Dept of Pediatric Hematology, Immunology & Infectious diseases, Emma Children’s Hospital, AMC, Amsterdam, Netherlands, 3Dept of Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands.

The biggest cause of death in children under 5 years consists of infection. The most common presenting symptom of infection is fever. Most of febrile illnesses are caused by viral infections at this age. However a small number are life-threatening bacterial infections, such as meningitis or pneumonia. In the clinic it may be difficult to distinguish between a bacterial or viral infection based on clinical grounds. This results in unnecessary treatment with antibiotics when they are suffering from a viral infection out of fear of missing a bacterial infection. There is an urgent need of the development of improved methods to distinguish between bacterial and viral infections. In a prospectively, we focused on identification of new discriminators of bacterial and viral infection. We performed a multiplex protein assay with 27 candidate markers and ELISA to validate known biomarkers. In 150 bacterial and viral plasma samples we have confirmed by ELISA that C-reactive protein and neutrophil protein elastase is increased in bacterial infections compared to viral infections. We found several pro-inflammatory proteins elevated in plasma from children with meningococcal infection. Also, neutrophil-derived S100A12 was significantly increased in bacterial infections, compared to viral infection. Further analysis into these markers is needed to get insight in the predictive value of these parameters. Our data clearly indicate that a reliable diagnostic tool based on a multiplex protein test will lead to a more accurate diagnosis to reduce hospital admissions and avoid overtreatment with antibiotics in febrile disease in pediatrics.

P.D4.02.01  
β-defensin as mast cell phenotype and activity modulator  
J. Agier1, S. Rozalska1, M. Wiktorska1, P. Zalewsksi1, E. Brzezińska-Blaszczyk1, T. Allen2,1,3, Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, 2Institute of Medical Virology, Zurich, Switzerland, 3Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland.

Background. Defensins play a crucial role as components of the early host defense against bacterial, viral and fungal invasion but new research has cast light on alternative immunomodulatory activities. Given that mast cells (MCs) are firmly efficient effector cells in microbial elimination and play an essential role in orchestrating inflammatory response during infection, this study analyzes hBD-2-induced expression of RIG-I, NOD1, and NOD2 receptors and evaluates the effect of this peptide on the pro-inflammatory response in vivo differentiated mature tissue MCs. Materials and methods. All experiments were carried out in vitro on freshly isolated peritoneal MCs obtained from female albino Wistar rats. qRT-PCR, flow cytometry, and confocal microscopy were used to evaluate both constitutive and hBD-2-induced expression of receptors. ROS was determined using H2DCFDA, and chemotaxis assay was used to define the MC migratory response. Standard procedure assessed histamine release. Results. hBD-2 enhances the expression and induces translocation of the studied receptors and directly activates the pro-inflammatory and migratory responses of native MCs. Conclusion. These data suggest that hBD-2 might augment MC capability and sensitivity to RLR and NLR ligands and strengthen the role of MCs in inflammation. Supported by the Medical University of Lodz (grant no 503/6-164-01/503-61-001).

P.D4.02.02  
Investigating metabolic mechanisms regulating collagen breakdown in tuberculosis  
R. M. Asher1, J. S. Friedland;1
1Imperial College London, London, United Kingdom.

Introduction: Tuberculosis (TB) is a global pandemic. Morbidity and mortality in TB result from inflammatory tissue destruction, driven by matrix metalloproteinases (MMP). Patients also experience profound weight loss. TB-infected macrophages display the Warburg effect, a metabolic shift from oxidative phosphorylation to aerobic glycolysis. However, the relationship between innate inflammation and cellular metabolism in TB remains poorly defined.

Methods: Primary normal human bronchial epithelial cells (NHBE) or monocyte-derived macrophages (MDM) were incubated with specific metabolic inhibitors, or transfected with siRNA. Cells were then directly infected with live, virulent TB or stimulated with conditioned media from TB-infected monocytes (CoMTB). Protein secretion, gene expression and functional tissue damage were measured by ELISA, luminex, zymography, real-time PCR and DG collagen assay.

Results: The glycolysis inhibitor, 2-deoxyglucose (2DG), reduced gene expression of the glycolytic enzyme, MMP-1, in a dose-dependent manner. There was a 7-fold drop in MMP-1 secretion in CoMTB-stimulated NHBE (p<0.0001) and a 5-fold drop in TB-infected MDM (p<0.0001). This was accompanied by a functional decrease in collagen breakdown. 2DG also decreased IL-1β (p<0.0001) and IL-10 (p<0.0001) and increased TNF-α (p<0.0012) in TB-infected MDM. Enhanced transcription factor HIF-1α expression in CoMTB-stimulated MDM was attenuated by 2DG. MMP-1 and IL-1β secretion were decreased by inhibiting the metabolic regulator AMPK and increased by Pi3-kine pathway blockade.

Conclusion: Our data show that glycolysis is a key modulator of MMP-1, cytokines and HIF-1α in TB. AMPK and Pi3-kine also have central regulatory roles. Research was funded by Medical Research Council (UK) and Mason Medical Research Foundation.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

Poster Presentations

P.D4.02.03
Plasma cytokines CCL2, CCL10 and IL18A correlate with disease severity in a controlled typhoid human challenge model
A. J. Barton1, M. Gibani2, E. Jones3, S. Camara4, Y. Rosenberg-Hasson1, J. Moharanu1, G. Obermoser1, J. Galan1, A. J. Pollard1
1University of Oxford, Oxford, United Kingdom, 2Human Immune Monitoring Center, Stanford University, Stanford, United States, 3Human Immune Monitoring Center, Stanford University, Stanford, United States, 4Yale University, New Haven, United States.

Introduction: Salmonella enterica serovar Typhi (S. Typhi) is a human-restricted pathogen estimated to cause 20 million cases of typhoid fever each year. The contribution of the typhoid toxin, a virulence factor specific to typhoidal Salmonella, to host-pathogen interactions in vivo is not well characterised. Here we investigate the changes in cytokine profile induced by wild-type and toxin-deficient S. Typhi challenge, and their correlation with disease severity.

Methods: 40 healthy volunteers were randomised to receive oral challenge wild-type Quaians strain 3. Typhi or an isogenic typhoid toxin-deficient mutant with an attack rate of 75% (90/40). Post-challenge changes in plasma cytokine profile over a two week time series were assayed using a 62-plex LumineX system. Raw intensities were normalised, and fold changes relative to baseline were compared using linear modelling.

Results: S. Typhi challenge induced a significant change in plasma cytokines when compared to the baseline (p<0.05). However, IL12 was significantly upregulated in the toxin-negative group but not in the wild-type group. CCL10 and IL18A were positively correlated with temperature (R = 0.9 and R = 0.8) and CCL9, CCL10 and IL18A with aggregete reported symptom scores.

Conclusion: Based on this extensive cytokine study, absence of the toxin may affect Th1 differentiation through IL-12. The correlation between disease severity and CCR5 ligands CCL9 and CCL10 could relate to their action on T cell migration, while endogenous antipycyotic IL1RA may have been upregulated to counteract the inflammatory action of IL-1.

P.D4.02.04
Anti-Cytomegalovirus IgG Antibody Titer is Positively Associated with Advanced T Cell Differentiation and Coronalary Artery Disease in End-Stage Renal Disease
Y. Chi1,2, K. Shu, I. Chen, F. Low3
1Far Eastern Memorial Hospital, New Taipei City, Taiwan, 2National Taiwan University, Taipei, Taiwan.

Background: Accumulating evidence indicates that persistent human cytomegalovirus (HCMV) infection is associated with several health-related adverse outcomes including atherosclerosis and premature mortality in individuals with normal renal function. Patients with end-stage renal disease (ESRD) exhibit impaired immune function and thus may face higher risk of HCMV-related adverse outcomes. Whether the level of anti-HCMV immune response may be associated with the prognosis of hemodialysis patients is unknown. Results: Among 412 of the immunity in ESRD study (ESRD study) participants, 408 were HCMV seropositive and were analyzed. Compared to 57 healthy individuals, ESRD patients had higher levels of anti-HCMV IgG. In a multivariate-adjusted logistic regression model, the log level of anti-HCMV IgG was independently associated with prevalent coronary artery disease (OR=1.93, 95% CI=1.2-3.2, p=0.01) after adjusting for age, sex, hemoglobin, diabetes, calcium phosphate product and high sensitivity C-reactive protein. Levels of anti-HCMV IgG also positively correlated with both the percentage and absolute number of terminally differentiated CD28+ and CD4+CD45RA+CCR7- TEMRA cells, indicating that immunosenescence may participate in the development of coronary artery disease. Conclusions: This is the first study showing that the magnitude of anti-HCMV humoral immune response positively correlates with T cell immunosenescence and coronary artery disease in ESRD patients. The impact of persistent HCMV infection should be further investigated in this special patient population.

P.D4.02.05
The potential role of Th17-like immune responses in Johne's disease positive cows
J. L. DeKuiper, P. M. Coussens
Michigan State University, East Lansing, United States.

Johne's disease (Jd) is a chronic gastrointestinal disorder of ruminants caused by Mycobacterium avium subspecies paratuberculosis (MAP). Later stages of Jd coincide with a classical Th2-like immune response. Defining importance of a classical Th1-like response in Jd has been more difficult. A possibility exists that non-classical responses, such as a Th17-like response, might be important in MAP immunity. Indeed, mRNAs encoding the cytokines IL-23 and IL-17A are significantly elevated in PBMCs from MAP test positive (Jd+), Jd test negative (Jd-) cows, and subclinical Jd+ cows (Jd+/-) after MAP antigen stimulation. Plasma from MAP test positive Jd+ cows (IL-17A and IL-12A) was performed and analyzed via flow cytometry to determine the relative proportion of IL-17+ cells. Using a combination of experimental data and mathematical models we now provide additional insights into mechanisms of these clusters. We show that a model in which clustering formation is driven exclusively by T-cell-extrinsic factors, such as variability in "attractiveness" of different MAP-infected cells, cannot explain the distribution of cluster sizes in different experimental conditions. In contrast, the model in which cluster formation is driven by the positive feedback loop (i.e., larger clusters attract more T cells) can accurately explain the available data. Mathematical modeling also suggested that formation of clusters occurs rapidly, within few hours after adoptive transfer of T cells, thus illustrating high efficiency of T cells in locating their targets in complex peripheral organs such as the liver. Taken together, our analysis provides novel information into the mechanisms driving the formation of clusters of antigen-specific CD8 T cells in this special patient population.

P.D4.02.06
Clustering of CD8 T cells around malaria-infected hepatocytes is rapid and is driven by antigen-specific T cells
V. V. Ganusov, R. Kelemen1, H. Rajakaruna1, J. Cockburn1
1Institute of Virology, Philipps University Marburg, Marburg, Germany, 2German Centre for Infection Research at the Institute of Virology, Philipps University Marburg, Marburg, Germany, 3Research Centre for Emerging Infections and Zoonosis, University of Veterinary Medicine, Hannover, Germany.

Malaria begins when Plasmodium-infected mosquitoes inject malaria sporozoites while searching for blood. Sporozoites migrate from the skin via blood to the liver, infect hepatocytes, and form liver stages. In mice, vaccine-induced activated or memory CD8 T cells are capable of locating and eliminating all liver stages in 48 hours, thus preventing the blood-stage disease. However, rules of how CD8 T cells are able to locate all liver stages in a limited timeframe remains poorly understood. We recently reported formation of clusters consisting of variable numbers of activated CD8 T cells around Plasmodium yoelii (Py)-infected hepatocytes. Using a combination of experimental data and mathematical models we now provide additional insights into mechanisms of these clusters. We show that a model in which cluster formation is driven exclusively by T-cell-extrinsic factors, such as variability in "attractiveness" of different Py-infected cells, cannot explain distribution of cluster sizes in different experimental conditions. In contrast, the model in which cluster formation is driven by the positive feedback loop (i.e., larger clusters attract more T cells) can accurately explain the available data. Mathematical modeling also suggested that formation of clusters occurs rapidly, within few hours after adoptive transfer of T cells, thus illustrating high efficiency of T cells in locating their targets in complex peripheral organs such as the liver. Taken together, our analysis provides novel insights into the mechanisms driving the formation of clusters of antigen-specific CD8 T cells in the liver.

P.D4.02.07
Innate recognition of heat-stable ligands from Orientia tsutsugamushi by C-type lectin receptors
V. Heffeler1, Z. Orfanos1, S. Mayer1, I. Chen2, S. Camara1
1Institute of Virology, Philipps University Marburg, Marburg, Germany, 2German Centre for Infection Research at the Institute of Virology, Philipps University Marburg, Marburg, Germany.

Orientia tsutsugamushi, an obligate intracellular Gram-negative bacterium causing the neglected febrile disease scrub typhus, elicits chemokine and cytokine production by heat-stable ligands via NF-kB-dependent pathways in phagocytes. It has not been studied how recognition by receptors other than Toll-like (TLR) and NOD-like receptors shape the inflammatory response to Orientia. Orientia has an atypical cell wall composition with high amounts of neutral saccharides in its outer membrane, which could predispose for recognition by C-type lectin receptors (CLR).

In order to screen for potential CLR ligands, we used purified heat-inactivated Orientia in a FACS-based interaction assay involving a library of 12 mouse and 4 human CLRs. We identified four mouse candidate receptors binding to Orientia. Binding to mouse Miclncle was EDTA-sensitive and thus shown to be specific. Miclncle was therefore chosen for further investigations.

Bone marrow-derived dendritic cells (BMDC) from C57BL/6 mice stimulated with inactivated Orientia showed an increasing, dose-dependent induction of miclnacle mRNA over 24 hours. Furthermore, upon stimulation significantly higher levels of TNF-a were seen in Miclncle-deficient BMDC compared to the C57BL/6 wildtype, suggesting an inhibitory effect of Miclncle on NF-kB-mediated cytokine production.

Combined, these results point toward an initial upregulation of Miclncle by another receptor, e.g. a TLR, before the former can mediate its inhibitory effect. We aim to provide further insight into the role of Miclncle in the recognition of Orientia tsutsugamushi.
POSTER PRESENTATIONS

P.D4.02.08

The role of ISG15 in proteasomal degradation and MHC class I antigen presentation

T. Held, M. Goetze,
Division of Immunology, Konstanz, Germany.

Interferon stimulated gene 15 (ISG15) is an interferon (IFN)-α/β-induced ubiquitin-like protein. It exists as an intracellular and extracellular molecule, as well as conjugated to target proteins (ISGylation). Both free and conjugated ISG15 exhibit antiviral activities against a wide range of viruses. Evidence indicates that ISGylation mostly targets newly synthesized proteins, as its E3 ligase Herc is physically associated with polyribosomes. It is suggested that upon viral infection newly translated viral proteins are primary targets of ISG15. Ubiquitinated and other ubiquitin-like proteins such as FA10 have been shown to be ISGylated. We provide evidence that ISGylation of viral proteins might as well contribute to proteasomal degradation of these proteins into MHC class I antigen presentation. To elucidate the role of ISG15 conjugation and proteasomal degradation, we treated interferon-induced as well as ISG15 overexpressing cells with the protein synthesis inhibitor cycloheximide and were able to show that ISGylation does not target proteins for proteasomal degradation. Thus, the biochemical function of ISG15 conjugation still needs to be further elucidated.

P.D4.02.09

Mechanism of induction of the Toll/Interleukin-1 receptor protein C (TcpC) of uropathogenic E. coli (UPEC)

J. Hemberger, T. Mietlicz,
Universitätsmedizin Mannheim, Mannheim, Germany.

Toll/Interleukin-1 receptor (TIR) proteins are present in many pathogens like uropathogenic Escherichia coli (UPEC). They interfere with the TLR-signaling chain, which is an essential part of the innate immune system. Toll/Interleukin-1 receptor protein C (TcpC) from the UPEC strain CFT073 is an essential virulence factor that impairs the innate immune system, i.e. TLRs and inflammasomes, increases the bacterial spread and causes severe organ damage. To find possible gene inducers we tested the native promoter of the operon that includes tcpC, a putative promoter directly in front of tcpC, and a segment that includes both promoters with gfpmut2 as a reporter gene that was measured by its fluorescence. We first explored whether different cell culture media influenced the promoter activity. The fluorescence of bacteria in DMEM ceased within 48 h while McCoy and RPMI caused an increase in fluorescence over 72 h. The overall fluorescence strength in McCoy and RPMI cultures was considerably higher as compared to relatively low signals in glucose-medium and EMEM. Transwell cultures were conducted to investigate if the induction is dependent on the bacterial density. We could show that an increasing bacterial density driven induction has a higher fluorescence of the reported promoter construct. This effect was stronger when the bacteria were incubated in McCoy medium. We then tested a different eukaryotic cell type as possible inducer, since UPECs should support their own immune response. RAWs did not have an influence on the putative promoter. However, more cells of the urogenital tract have to be tested.

P.D4.02.10

Altered IL-12/IFN-γ pathway in extrapulmonary tuberculosis and visceral leishmaniasis in pediatric patients and related controls

A. Esteve-Soler\(^1\), A. Deyo-Martínez\(^2\), A. Noguera-Julian\(^3\), A. Martín-Naldí\(^4\), E. Cobo\(^5\), C. Fortuny\(^6\), R. Soler-Palacios\(^7\), L. González-Granada\(^8\), C. Gionetti\(^9\), M. Córdoxa\(^10\), M. Antón\(^11\), V. Belchí\(^12\), J.L. Barceló-Docto\(^12\), J. Iglesias\(^12\), A. M. Plaza\(^12\), M. Juana\(^12\), L. Alsina\(^12\).

\(^1\)Pediatric Allergy and Clinical Immunology Department. Hospita Sant Joan de Déu, \(^2\)Instituto de Investigación en Pediatría Hospital Sant Joan de Déu, \(^3\)Hospital Universitario San Cecilio, \(^4\)Hospital Universitario de Granada, \(^5\)Hospital Universitario Dr. Peset, \(^6\)Hospital Clinic-IDIBAPS, \(^7\)Hospital Universitario Ramón y Cajal, \(^8\)Hospital Universitario La Fe, \(^9\)Hospital Universitario Universitat de Valencia, \(^10\)Hospital Universitario La Paz, \(^11\)Hospital Universitario Gregorio Marañón, \(^12\)Universitat de Girona.

The regulation of metabolism in immune cells or virally infected cells has been well studied. However, the precise metabolic regulation that ensues when both immune system and pathogen co-exist is not fully understood. In this study we aimed to investigate the integrative role of metabolism in immune cells or virally infected cells to understand how a better metabolism leads to an enhanced immune response. In this study, we have analyzed the transcriptome and metabolome of wild-type and IFNαR-/- or IFNβR-/- macrophages infected with productive and non-productive (attenuated) MCMV strains. This allows unraveling host versus virus directed metabolic alterations observed upon infection of macrophages. We find that MCMV takes advantage of the early inflammatory metabolomic reprogramming of activated macrophages to establish infection in the cells.

P.D4.02.11

Viral co-option of IFN driven glycolytic programming in infected macrophages

K. Kotsamanis\(^1\), J. Edwards-Hicks\(^2\), A. Alghamdi\(^3\), P. Larozet\(^4\), M. Blanc\(^5\), D. G. Watson\(^6\), A. Finch\(^7\), P. Ghazali\(^8\).

\(^1\)University of Edinburgh, Edinburgh, United Kingdom, University of Strathclyde, Glasgow, United Kingdom.

Immunity and metabolism have been viewed as separate fields, however, recent evidence show that these two systems are intimately integrated, share resources and cross-regulate each other. Activated immune cells alter their metabolism in order to support effector functions. On the other hand, viruses are obligate parasites that counter and exploit host pathways, including metabolism in infected cells, to effectively propagate. The regulation of metabolism in immune cells or virally infected cells has been well studied. However, the precise metabolic regulation that ensues when both immune system and viral infection, in immune cells, interact and compete for the limited resources and available metabolic pathways is not clear. Here, we have sought to investigate this integrative process by studying the metabolic programming of macrophages infected with murine cytomegalovirus (MCMV). Our hypothesis is that productive infection of macrophages by MCMV takes advantage of the early inflammatory metabolic reprogramming of activated macrophages to establish infection in the cells. To study this interaction, we have analyzed temporally the transcriptome and metabolome of wild-type and IFNβR-/- or IFNαR-/-. MCMV infected with productive and non-productive (attenuated) MCMV strains. This allows unravelling host versus virus directed metabolic alterations observed upon infection of macrophages. We find that cytomegalovirus co-opts early pro-inflammatory changes in glycolysis for establishing infection of macrophages. This represents a novel previously unappreciated host pathogens interactions pathway.

P.D4.02.12

Murine neutrophils modulate adaptive immunity during brucellosis

R. Mara-Cartel\(^1\), C. Gutiérrez-Jiménez\(^1\), A. Alfaro-Alarcón\(^1\), E. Chaves-Ortiz\(^1\), E. Barquero-Calvo\(^1\), E. Moreno\(^2\).

\(^1\)Programa de Investigación en Enfermedades Tropicales, Universidad Nacional, Heredia, Costa Rica; \(^2\)Departamento de Patología, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica, \(^3\)Centro de Investigación en Enfermedades Tropicales, Universidad de Costa Rica, San José, Costa Rica.

Polymorphonuclear neutrophils (PMNs) are part of the first line of defense against microbial pathogens. We have previously demonstrated that PMNs negatively influence the Th1 immune response at early times of Brucella infection (PLOS Pathog 9: e1003167). To investigate the influence of PMNs in the adaptive immune response during chronic brucellosis, we exploited these leukocytes by means of antibodies against PMNs. During the course of the infection, we have demonstrated that at later times of Brucella abortus infection, the bacterium is killed more efficiently in the absence of PMNs than in their presence. Removal of neutrophils during infection decreased spleen inflammation, induced elevated production of INF-γ, IL-6, IL-12 and IL-10, and caused transient cachexia. The absence of PMNs during Brucella infection caused a decrease in most antibody isotypes against B. abortus. The only exception was the increase of IgG3 isotype, an event linked to the high amounts of IFN-γ produced in neutropenic mice. These results reveal that late removal of PMNs have an unexpected influence in modulating the immune response against brucellosis.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 475
Moreover, it remains poorly understood why most infected neonates remain asymptomatic whereas some show moderate or severe disease symptoms. Here, I aim to investigate the interaction networks associated to HCV Core protein. Moreover, in order to identify proteins associated with HCV Core, in Jurkat T cells, we carried out alterations caused by this viral protein, we have analyzed HCV Core subcellular localization and its associations with host proteins in Jurkat T cells. We performed immunogold electron microscopy techniques to analyze the subcellular localization of Core protein. Moreover, in order to identify proteins associated with HCV Core, in Jurkat T cells, we carried out pull-down assays combined with Mass Spectrometry Analysis. Thus, in this work, we show the ultrastructural localization of HCV Core in Jurkat T cells and the host molecular interaction network associated to HCV Core protein.
Canine visceral leishmaniasis (CVL) is a major health issue in many tropical and sub-tropical countries and an accurate diagnosis still remains a challenge. The present study aimed to compare the diagnostic accuracy of rKL0B and rK28 in discriminating canine visceral leishmaniasis from Leishmania donovani vaccinated dogs

1Federal University of Juiz de Fora, Juiz de Fora, Brazil, 2Santa Agostina Veterinary Hospital, Belo Horizonte, Brazil, 3Philips University of Marburg, Marburg, Germany, Infectious Disease Research Institute, Seattle, United States.

Canine visceral leishmaniasis (CVL) is a major health issue in many tropical and sub-tropical countries and an accurate diagnosis still remains a challenge. The present study aimed to compare the diagnostic accuracy of rKL0B, a new antigenic kinesin-related protein of Leishmania donovani, and rK28, a recombinant fusion polypeptide comprising rKL26, rKL29 and an antibody against Leishmania chagasi. Uninfected whole antigen was also investigated. Sera samples from diseased dogs (CVL, n=44) and healthy endemic controls (EC, n=44) were evaluated for the presence of antigenic IgG, IgG1 and IgG2 antibodies by ELISA. The sensitivity and specificity of each ELISA was investigated by ROC curve analysis. Antigen-specific reactivity was also tested against sera from Leish-Tec®-vaccinated dogs. Enhanced levels of IgG and mainly IgG2 to both rKL0B and rK28 were found in diseased dogs. The ELISAs using rKL0B and rK28 showed a sensitivity of 77% and 84% for IgG and 78% and 88% for IgG2, respectively. A specificity of 94% for rKL0B and 95% for rK28 was observed using IgG2 ELISA, and specificity of 100% for both antigens was observed using IgG1 ELISA. rK28 was the only antigen able to demonstrate differences between vaccinated and infected groups (p < 0.001), but showing no difference between vaccinated and control (EC) groups. These results indicate the usefulness of rKL0B and rK28 in the serodiagnosis of CVL, and suggest that rK28 does not cause a more diagnostic confusion which can be applied to vaccinated dogs. Financial support: CNPq and FAPEMIG, Brazil.

P.D.4.02.19
Complement factor H family proteins associate with severity of bacterial infections in children
A. E. van Beek1, N. A. Schweintzer2, R. B. Pouw1, D. S. Klo bassa2, M. C. Brouwer1, J. Geissler2, A. Bieß2, M. Sagnermeier3, D. Wouters4, W. Zemt5, T. W. Kuipers1,6,7, the EUCLIDS consortium;
1Department of Infectious Diseases, Sanquin Research and Landsteiner Laboratory of the Academic Medical Center, Amsterdam, Netherlands, 2Department of Pediatric Hematology, Immunology and Infectious Diseases, Emma Children’s Hospital, Academic Medical Center, Amsterdam, Netherlands, 3Department of General Pediatrics and Adolescent Medicine, Medical University of Graz, Graz, Austria, 4Department of Pathology, Sanquin Research and Landsteiner Laboratory of the Academic Medical Center, Amsterdam, Netherlands, 5Department of Pediatrics, Kepler University Clinic, Medical Faculty of the Johannes Kepler University, Linz, Austria.

Introduction: Complement is part of the innate immune defense against invading pathogens. Concurrent protection from complement is acquired by Factor H (FH), although this protection is thought to be inhibited by FH-related proteins (FRHs). As pathogens recruit FH from human plasma as escape mechanism to increase survival in blood, binding of FH instead of FH might prove beneficial for clearing an infection. However, little is known about the plasma levels of these FH family proteins during invasive bacterial infections. Methods: We included pediatric patients with acute invasive bacterial infections as part of the EUCLIDS study, together with age-matched healthy controls. In-house ELISAs were used to determine FH and FRH plasma levels.
Results: FH levels were low during the acute phase and associated strongly with severity. Similarly, FHR-2 and FHR-4A levels were low in the most severe patients. In contrast, FHR-5 levels were elevated during the acute phase, irrespective of severity or causative microbe, although levels were generally low in patients who suffered from meningococcal disease.
Conclusions: Our study shows that plasma levels of FH family proteins associate with severity of invasive bacterial infections in children. Moreover, we found indications for a novel role for FH-related proteins. Further studies are needed to confirm the role of FH-related proteins in infectious diseases. A better insight into their level and function during invasive infections will help to improve critical care for pediatric patients.

P.D.4.02.20
Respiratory Syncytial Virus directly infects natural killer cells and affects anti-viral effector functions
E. A. van Erp1, D. Feyaerts2, M. Duijst3, L. H. Mulder4, O. Wich5, W. Luyp6, G. Ferwerda7, P. B. van Kastereni;
1National Institute for Public Health and the Environment, Bilthoven, Netherlands, 2Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands.

Respiratory syncytial virus (RSV) infection can lead to severe respiratory illness and is the main cause of hospitalization in infants under 1 year of age. No vaccines or antivirals are currently available and the determinants of severe disease remain elusive. Natural killer (NK) cells are important effector cells in the anti-viral immune response and likely form a critical component of the early response against RSV infection. NK cells that are recruited to the lung during RSV infection encounter the virus in the presence of maternal antibodies. We investigated whether RSV and RSV-antibody complexes affect NK cell functionality, since these cells potentially contribute to immunopathology. We demonstrate for the first time that RSV can directly infect adult and neonatal NK cells. Incubation of RSV with sub-neutralizing antibody concentrations significantly increased the percentage of infected NK cells, and this increase was FcγRII/CD16 dependent. Upon infection, large numbers of NK cells produced IFN-γ, but this was not accompanied by enhanced killing capacity as determined by the percentage of perforin-secreting cells. RSV-infected NK cells therefore appear geared towards a pro-inflammatory rather than a cytotoxic response. Our findings show that RSV can affect NK cell functionality, an effect that is enhanced in the presence of sub-neutralizing antibody concentrations. Considering that NK cells that are currently being developed aim at inducing (maternal) antibodies against RSV, it is extremely important to have a good understanding of the possible interactions between innate effector immune cells and virus-specific antibodies and their role in the development of (severe) RSV disease.

P.D.4.03 Exploiting host pathogen interaction - Part 3
P.D.4.03.01
Characterisation of the effects of interleukin-17A (IL-17A) on toll-like receptor 3 (TLR3)-function and its role in accelerating disease progression in human idiopathic pulmonary fibrosis (IPF)
M. E. Armstrong1, L. Bergin1, A. N. McElroy2, G. Coke3, P. G. Follain4, C. M. Hogaboam5, N. Hizan6, S. D. Connely7;
1Trinity Biomedical Sciences Institute, Dublin 2, Ireland, 2Institute of Technology Tallaght, Dublin 24, Ireland, 3Cedars-Sinai Medical Centre, Los Angeles, United States, 4University of Edinburgh, 5Trinity College Dublin, 6NUI Galway, 7University of Limerick, Limerick, Ireland.

Introduction: In this study, we investigated the ability of IL-17A to modulate TLR3 function in primary lung fibroblasts from patients with idiopathic pulmonary fibrosis (IPF). Using a case-control study, we also investigated the role of the IL-17A promoter-polymerisation, IL-17A G197A (rs2273914) in the development of IPF. Previously, our laboratory demonstrated that defective TLR3 function was associated with a significantly greater risk of mortality and an accelerated rate of decline in lung function, in IPF patients [ARCCM; 181(8):1442-50]. We additionally detected increased levels of IL-17A in bronchoalveolar lavage fluid and lung tissue from IPF patients [PNAS; 111(367-72)]. Materials and Methods: Primary human lung fibroblasts from IPF patients were treated with Poly(C:U) in the presence or absence of IL-17A. Cytokine, chemokine and type I interferon levels in IPF fibroblasts were determined by ELISA and qPCR, respectively. Patients with IPF were additionally genotyped for the IL-17A G197A polymorphism. Results: We established that IL-17A can modulate TLR3-function in IPF lung fibroblasts to reduce production of the anti-viral mediators, IFN-β and RANTES. Concomitantly, IL-17A can also synergistically increase TLR3-induced IL-8 from IPF lung fibroblasts. Using a case-control study for IPF, we also demonstrated that individuals who are homozygous for the variant A allele of the IL-17A G197A promoter-polymerisation, are significantly more at risk of developing IPF. Conclusions: These results support a novel role for IL-17A in promoting disease progression in IPF via its modulation of TLR3 function in IPF fibroblasts. In addition, this study reveals IL-17A G197A as a candidate biomarker in IPF.

P.D.4.03.02
In silico identification of Taenia solium secrectory secretory protein and it’s suppressive activity for PI3K/AKT pathway
N. Arora, A. Prasad;
Indian Institute of Technology Mandi, Mandi, Himachal Pradesh, India.

Objective: Taenia solium is a parasitic infection of central nervous system, causing neurocysticercosis (NCC). Parasite secrectory products modulate host immune response. To utilize for FNHR-5 as acute phase protein in human with parasitic infection, excretory secretory proteins will be needed to further clarify the role of these proteins in the infection. The objective was to predict T. solium secretory proteins that are related with AKT pathway and validate it with LC-MS and wet lab tools. Material & Methods: Cysts were isolated from infected swine and cultured for 24Hrs. The ES-proteins (30ug) was prepared for LC-MS analysis by in-gel trypptic digestion. LC-MS spectra was obtained and annotated. Simultaneusly, in silico T. solium ES-proteins were predicted using tools TargetP, SignalP, SecretomeP and THMM. Blast2GO was used to perform BlastP, protein annotation and KEGG mapping. After comparing the LC-MS and predicted annotations, PI3K regulatory subunits were identified in both the pathways in response to ES proteins in human primary macrophages. Results: The T. solium proteome consist of ~12000 proteins, ~1000 were predicted as ES-proteins and 355 mapped to global metabolic pathways of which 8 were related with PI3K pathway. LC-MS analysis of in vitro cultured T. solium ES-proteins annotated 30 proteins and two were related with PI3K regulation. The cells treated with ES proteins had significantly less activation of pAKT compared to untreated cells. Conclusion: ES proteins are important for immune modulation of host by T. solium, and ES-proteins target PI3K/ AKT pathway by down regulating it and thus suppressing the immune response.
Antirretroviral treatment (ART) of primary HIV infection (PHI) has demonstrated virological and immunological benefits. The effect of early ART during PHI on the level of growth factors and chemokines modulating immune cell functions remain to be established. The aim of our work was to analyze the dynamics of 27 cytokines (pro-inflammatory, Th1, Th2), chemokines (IL-8, CCL2, CXCL9, MCP-1, MIP-1b, RANTES) and growth/regulation factors (TRAIL, SCF, SCGF-b, HGF, M-CSF, G-CSF, GM-CSF, LIF) in plasma of HIV infected patients treated during PHI. Patients with PHI (n=43) were enrolled before, 24 and 48 weeks after therapy initiation. Omission of soluble immune mediators described above was evaluated in HIV infected patients and healthy donors (HD, n=9) by Luminox technology. The cytokines profile was strongly perturbed in primary HIV infected patients when compared to HD. After 48 weeks of ART, some of these factors were restored to HD level (IL-7, IL-9, LIF) while others persisted higher than HD (IL-6, IL-10, G-CSF). Interestingly, a subset of chemokines, such as MCP-1, IL-8, MIP-1b, RANTES and CCL2, as well growth factors such as HGF, SCF and GM-CSF, increased during ART reaching values significantly higher than HD after 48 weeks. The increase of chemokines with antiviral activity and of growth factors with hematopoietic and immunomodulatory properties may have a beneficial effect. Other studies are mandatory to evaluate the long lasting effects of these factors to clarify their possible role in the context of protection/pathogenesis.

Stimulation of MAC-inhibitory protein (CD59) but no complement decay-accelerating factor (CD55) induces release of neutrophil extracellular trap (NETs)

We conclude that RNS efficiently stimulate NETs formation and PI3K activity, but not autophagic flux, is necessary for this process. PI3K influences NETs formation via regulation of release and the synthesis of reactive oxygen species (ROS) were assessed by fluorometry and fluorescent or light microscopy. Autophagy was analyzed by western blotting as poorly understood. Our aim was to investigate RNS as NETs stimuli and to identify the role of autophagy in this process.

Introduction: MAC-inhibitory protein, also known as CD59 is a membrane inhibitor of reactive lysis found on cell surface of leukocytes and erythrocytes. When complement activation leads to deposition of C5b678 on host cells, CD59 can prevent C9 from polymerizing and forming the complement membrane attack complex. CD55 recognizes C4b and C3b fragments that are created during activation of C4 (classical or lectin pathway) or C3 (alternative pathway) thus indirectly blocks the formation of the membrane attack complex. CD59 and CD55 attach to host cells via a glycosphosphatidylinositol (GPI) anchor. A mutation of PIG-A gene, which leads to deficiency of GPI, is found in patient with paroxysmal nocturnal hemoglobinuria. The aim of the study was to establish, if release of neutrophil extracellular traps might be dependent on CD59 or CD55 activation. Materials and methods: Neutrophils were isolated from the blood of healthy donors. Cells were obtained by density gradient centrifugation and subsequent polyvinyl alcohol sedimentation of plasma. Neutrophils were cultured, then human recombinant or anti-CD55 monoclonal antibody and 100 nM PMA was added to stimulate NETs release. The process of NETs release was assessed 3h post stimulation by fluorescent microscopy and Fluorometry. Results: Anti-CD59 antibody induced NETs release in a concentration-dependent manner, with the highest release at 0.25 - 1.25 μg/mL. Fluorescent microscopy confirmed results of quantification method. None of studied anti-CD55 monoclonal antibody concentration lead to release of NETs. Conclusion: Neutrophil extracellular traps release is dependent on activity of GPI anchored proteins which regulates complement activation pathway.

The role of Fas/FasL receptors in pathogenesis of the inflammation of the nervous system induced by HSV-1 and 2 infection

Introduction. The aim of this project was to determine the role of Fas/FasL receptor signalling in pathogenesis of the inflammatory lesions occurring during herpes simplex virus type 1 and 2 (HSV-1/2) infection of the central and peripheral nervous system. Materials and methods. In vitro models consisted of mixed glial culture, primary microglia culture obtained from C57BL/6 mice. In vivo studies used intranasal model of C57BL6 mice infected with HSV-1 McKrae strain, and genital model of C57BL6 mice infected with HSV-2 333 strain. Apoptosis, Fas and Fas expression and phenotype of immune competent cells were accessed using confocal microscopy and flow cytometry. Results. Upon HSV-1 or HSV-2 infection, microglia underwent early apoptosis and up-regulated Fas expression, while HSV-1/2 infected astrocytes also up-regulated FasL and were resistant apoptosis. Both microglia and astrocytes were resistant to Fas-induced apoptosis. However, stimulation microglia became M1-type cells and switched the profile of produced cytokines. In vivo, Fas expression was detected on astrocytes surrounding infected sites in spinal cord (HSV-2) and brain (HSV-1) as well as on microglia within glia limitans and infected neuronal tissue. Fas positive cells were mostly infiltrating lymphoidal cells. Conclusion. We found that there is a correlation between HSV infection and reaction of cells to Fas/FasL induced apoptosis. Fas/FasL signalling can participate both in direct elimination of HSV infection, but also in a complex regulation of the local inflammatory response and mounting of the specific anti-viral response through non-apoptotic signalling. This work was supported by 2015/18/M/NZ6/00414 grant.

Lack of Polyfunctional Cytomegalovirus-specific T cells in Hemodialysis Patients

Background: Polyfunctional T cells are critical for maintaining protection against pathogens. Patients with end-stage renal disease (ESRD) are at increased risks for infectious complications and their T cell immunity against viruses may be impaired. The current study intends to investigate T cell immunity in ESRD patients by analyzing T cell differentiation and polyfunctionality against cytomegalovirus (CMV), an ubiquitous pathogen. Method: 21 healthy individuals, 13 patients with chronic kidney disease (CKD) and 47 ESRD patients were enrolled in this study. The cellular frequencies of CMV-reactive CD4+ and CD8+ T cell were comparable, patients treated during PHI. Patients with PHI (n=43) were enrolled before, 24 and 48 weeks after therapy initiation. All donors were seronegative for CMV. Two CMV peptide pools (E1 and pp65) were used to stimulate PBMCs and four effector functions were measured by multicolor flow cytometry (IL-2, TNFα, IFNγ and CD107a) to identify polyfunctional cells. Result: Age of the three groups was similar (mean, 60 years old). Patients with renal disease, especially ESRD patients, showed increase levels of erythrocytes. Subsequently, neutrophils were incubated with human antiCD59 or antiCD55 monoclonal antibody and 100 nM PMA was added to stimulate NETs release. The antibody concentration lead to release of NETs. Conclusion: Neutrophil extracellular traps release is dependent on activity of GPI anchored proteins which regulates complement activation pathway.

IMPACT OF ART ON DYNAMICS OF GROWTH FACTORS AND CYTOKINES IN PRIMARY HIV INFECTION

V. Bordoni, A. Sacchi, R. Cazetti, E. Cimini, C. Pinetti, A. Mondi, A. Ammassari, A. Antonini, C. Agrati; INMI L.Spallanzani, Rome, Italy.

Crosstalk of autophagy and reactive oxygen species synthesis in reaction to reactive nitrogen species-induced neutrophil extracellular traps formation

A. Manda-Handzik1,2, 3, M. Wachowski1, W. Bystrzycka1, O. Ciepiela1, U. Dzemkow1; 1Medical University of Warsaw, Warsaw, Poland, 2Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Warsaw, Poland.

Autophagy is a natural, self-degradative process which regulates neutrophil antimicrobial functions. One of the neutrophils' antimicrobial strategies is the formation of neutrophil extracellular traps (NETs). Although mechanisms of NETs formation are extensively studied, some aspects of this process, e.g. the role of reactive nitrogen species (RNS), remain poorly understood. Our aim was to investigate RNS as NETs stimuli and to identify the role of autophagy in this process. Human blood neutrophils were stimulated with nitric oxide (NO) donor - SNAP (S-Nitroso-N-acetylpenicillamine), peroxynitrite or phosphor bil-2-myristate 13-acetate (PMA). NETs release and the synthesis of reactive oxygen species (ROS) were assessed by Fluorometry and fluorescent or light microscopy. Autophagy was analyzed by western blotting as accumulation of LC3-II protein. We found that contrary to PMA, RNS stimulate NETs release without accumulation of LC3-II. Inhibitors of class III PI3 kinases (3-methyladenine, 3-MA, and wortmannin, used as inhibitors of autophagy), but non-inhibitors of the autophagosome formation, drastically reduced NETs formation upon RNS treatment. RNS only slightly increased ROS production by neutrophils, but 3-MA and wortmannin significantly decreased ROS production by RNS-stimulated neutrophils. Finally, we found that activity of NADPH oxidase was necessary for SNAP-induced NETs release and contributed to peroxynitrite-induced NETs formation. We conclude that RNS efficiently stimulate NETs formation and PI3K activity, but not autophagic flux, is necessary for this process. PI3K influences NETs' formation via regulation of NADPH oxidase activity. Acknowledgements This work was supported by the National Science Centre, Poland (Preliminary 2015/19/N/NZ6/0317) and the Foundation for Polish Science (POWR07/2016/2-7).
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P.D4.03.08
Functional roles of atypical IκB family members in macrophages
A. Matthies, K. Katsoulis-Dimitriou, C. Plaza Sirvent, J. Schmitz
1Helmholtz Centre for Infection Research, Braunschweig, Germany; 2Otto von Guericke-University, Magdeburg, Germany.

The activation of the transcription factor NF-κB is regulated by inhibitor of NF-κB proteins (IκBs), which include not only classical cytoplasmic proteins such as IκBα, but also atypical nuclear proteins such as Bcl-3, IkBε and IkBζ. Notably, the functions of the atypical IκB proteins in macrophages as well as their interactions remain poorly understood. To address this, we tested whether the atypical IκB proteins Bcl-3, IkBε and IkBζ in macrophages exhibit interdependent regulation of their expression and function. Our results showed that the atypical IκB proteins are expressed in primary bone-marrow-derived macrophages by Toll-like agonist with different kinetics. Interestingly, following LPS stimulation of RAW 264.7 macrophages, IkBε and IkBζ are upregulated early on, whereas Bcl-3 is upregulated at later time points. To analyze the functional relevance of IκB expression in macrophages, we infected wildtype and IκB-deficient macrophages with GFP-expressing Staphylococcus aureus in vitro. In preliminary experiments, we detected no alterations of intracellular bacteria in IκB-deficient macrophages compared to wildtype controls suggesting that IκB deficiency does not affect the phagocytosis capacity of macrophages. Furthermore, we found no difference in proliferation of intracellular S. aureus of IκB-deficient macrophages revealing that IκB deficiency does not alter the killing activity of macrophages. To extend this analysis also in in vivo experiments, we generated lysM-Cre,IkBε+/−,IkBζ−/− mice that lack IκBε specifically in macrophages and neutrophils. An initial immunophenotyping revealed no alterations in frequencies or absolute numbers of macrophages, neutrophils and dendritic cells suggesting that IκBζ is dispensable for the development of these cells.

P.D4.03.09
Modulation of T-cell cytokine profiles by (killer) B-cells during Tuberculosis disease
D. K. Moore, I. C. Van Rensburg, A. G. Loxton; Stellenbosch University, Cape Town, South Africa.

Rationale: Emerging evidence has implicated B-cells as important players in the defense against Mycobacterium tuberculosis. This study aimed to identify potential mechanisms by which B-cells may modulate T-cell function, as they have been regarded as the main immune cells involved in eradicating TB disease. Method: T-cells were cultured with BCG-exposed or naïve B cells that had been pulsed with or without CD40L and IL-12, to induce a regulatory T-cell sub-type. These killer B cells influence functional activity of T cells and are still unclear. Results: Killer B cells influence functional activity of memory CD4+ T cells following successful TB treatment. This suggests a role of B cells in protective immune responses to TB disease. An increase in the frequency of effector T-cells, although non-significant, was observed in healthy Mtb exposed individuals following culturing with unstimulated B cells and a decrease following culture with BCG-stimulated B-cells. This may imply a role of B-cells in T-cell function through regulation of phenotypic frequencies. Finally, B-cells that were CD40L/IL-5 pulsed on day 1 and produced cytokine production by both CD4 and CD8 T cells in healthy exposed and unexposed individuals. However, these alterations in cytokine profiles were not significant. Conclusion: B-cells and killer B cells influence T-cell behavior by modulating phenotype development and cytokine secretion. These results suggest a key role of Breg in initiating and guiding the immune response against Mtb.

P.D4.03.10
Multiparameter analysis of association between host immune reactivity and pulmonary tuberculosis activity

Tuberculosis (TB) is a highly contagious infectious disease characterized by different outcomes. Both impaired and exacerbated immune responses contribute to TB pathogenesis. However, the exact role of immunological hypo- and hyper-reactivity in TB pathology and their biomarkers are unknown. We analyzed how various indicators of innate and adaptive immunity are associated with TB severity. Sixty four TB patients (TBP) and 28 healthy contacts (HC) were included in the study. In each participant, we performed Quantiferon-TB Gold Plus assay and analyzed 46 immune factors in serum and antigen-induced plasma using XMAP multiplex assay. In TBP, the degree of pulmonary destruction, TB extent, systemic intoxication and bacteria excretion were accurately evaluated.

TB and HC could be well discriminated based on the levels of IL-2, IFN-γ, IP-10 and IL-8 in antigen-induced plasma (p<0.005) and GM-CSF in serum (p<0.0001). Analysis of immunological analytes in antigen-induced plasma of TB-identified 14 factors that discriminated TB into immunologically "hypo-" and "hyper-reactive" groups. The factors included type I and III interferons, members of IL-10 and IL-12 cytokine superfamilies. Based on the level of IP-10 in serum, TB clustered into other two groups. High HP-10 levels (>730 pg/ml) were indicative of severe pulmonary destruction (p<0.05) and predicted its slow/unfavorable dynamics. In conclusion, we identified factors that discriminated TB groups with high and low immune reactivity, which may be helpful for personalized implementation of pathogenetic therapy. IP-10 can serve as a candidate biomarker of TB infection activity and patients' responsiveness to anti-TB treatment.

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P.D4.03.11
Evaluation of interferon-gamma role during zika virus murine experimental infection
C. M. Polonio, N. Zanqui, L. Oliveira, C. Longo, J. Peron; Neuroimmune Interactions Laboratory - Department of Immunology - University of São Paulo, São Paulo, Brazil.

The flavivirus Zika (ZIKV) was recently introduced in Brazil causing an alarming increase of babies born with microcephaly. Genetic differences, mainly related to Interferons (IFN), and 3 days post infection. Our results demonstrated an important role of the IFN-γ in suppressing ZIKV infection, since IFN-γ induced cytokine production by both CD4 and CD8 T-cells in healthy exposed and unexposed individuals. However, these alterations in cytokine profiles were not significant. Conclusion: B-cells and killer B cell influence T-cell behavior by modulating phenotype development and cytokine secretion. These results suggest a key role of Breg in initiating and guiding the immune response against Mtb.

P.D4.03.12
Modulation of T-cell cytokine profiles by (killer) B-cells during Tuberculosis disease
A. Prasad, N. Aora; School of Basic Sciences, Indian Institute of Technology Mandi, Mandi, HP, India.

Objective: Larvae of Taenia solium cause Neurocysticercosis (NCC), which is most widespread cause of acquired epilepsy in developing countries. Excretory/secretory (ES) proteins released by larvae of T. solium are crucial for parasite survival and represent potential targets for novel intervention strategies. The current study was carried out to immune characterise ES proteins of T. solium. Method: Cysts were isolated from naturally infected pork muscles and cultured in RPMI-1640 complete media for 24 hours. ES proteins were characterized by silver staining, 2D-NMR, LC-MS spectroscopy and enzyme electro immune transfer blot (EITB) with NICC patients serum. Human macrophages isolated from buffy coat were stimulated with the ES proteins for 24hrs to look at their immune cell stimulating capabilities. Results: NMR spectra showed a number of metabolites being excited by the cyst. We identified several bands of <50kDa on EITB. LC-MS analysis of in vitro cultured ES protein annotated 107 proteins and two were related with PI3K regulation.ELISA and ELISA had shown significantly low IL6, IL1band enhanced IL4 cytokines expression. Conclusion: The ES proteins of T. solium suppress the Th1 immune response and help in parasite survival in host.
Complement factor H-related protein 1 impairs factor H acquisition during complement evasion by the malaria parasite Plasmodium falciparum

T. Reiß1, T. S. Rosã, R. P. Bobbert1, P. S. Zipfel2, C. Skerka2, G. Padeuf1
1RWTH Aachen University, Aachen, Germany; 2Hanns-Knoll-Institute, Jena, Germany.

Human complement is the first defense line against invading pathogens, including the unciliated malaria parasite Plasmodium falciparum. We previously demonstrated that human complement represents a threat for the clinically relevant blood stages of the parasite. To evade complement-mediated destruction, these acquire factor H (FH) via specific receptors, resulting in inactivation of complement factor C3b. We now report that the FH-related protein FHR-1 competes with FH for binding to the malaria parasite. FHR-1, which is known to mediate innate immune responses and is a conventional repeat domain protein, binds to FH but which lacks the C3b regulatory activity, accumulates on the surfaces of the intraerythrocytic schizonts as well as of free merozoites. While binding of FH to schizont-infected red blood cells is increased in FHR-1-deficient human serum, the addition of recombinant FHR-1 decreases FH-binding and in consequence parasite viability. We conclude that FHR-1 acts as a modulator of human immunity by counteracting FH-mediated microbial complement evasion.

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Immune regulation by myeloid cells leads to protection of Plasmodium berghei ANKA-infected kio mice from experimental cerebral malaria

J. Vitallé1, M. J. Menster1, J. M. Kuepper1, J. F. Scheunemann1, J. J. Reichwald1, A. Mueller1, A. Hoerauf1, D. R. Engel1, B. Schumak1
1Institute of Medical Microbiology, Immunology and Parasitology, University of Bonn, Bonn, Germany; 2Centre of Infectious Diseases, Parasitology Unit, Heidelberg University Hospital, Heidelberg, Germany; 3DZIF, Partner Site Bonn-Cologne; 4Department of Immunodynamics, University Essen Duisburg, Essen, Germany.

Inflammatory responses aim at pathogen elimination but need tight control as excessive immune activation can cause severe host-induced pathology. Cerebral malaria is a fatal complication of Plasmodium falciparum infection and an important example for overwhelming Th1-driven inflammation. This disease can be studied with the help of experimental models. Whereas wildtype (WT) mice develop experimental cerebral malaria (ECM) upon infection with Plasmodium berghei ANKA (PbA), transgenic mice that lack type I interferon receptor (Ifnar) dependent signalling are protected from ECM. Using transgenic Plasmodium berghei ANKA parasites expressing ovalbumin (PbA-OVA), we show that ECM-protected PbA-infected Ifnar1−/− kio mice did not differ in their antigen-specific cytokotic T cell responses compared to infected WT mice suffering from ECM. Importantly, spleens of ECM-negative Ifnar1−/− mice contained increased numbers of both CD8+ T cells and distinct myeloid cells, including M2 macrophages, confirmed by expression of typical markers such as RELMα and YM1 as well as their function shown by elevated arginase activity. Furthermore, in vitro coculture experiments demonstrated that individual myeloid cells derived from bone marrow were capable to exhibit suppressive capacities. We conclude that type I IFN signalling is not required for the generation of antigen-specific T cells in PbA-infected [Ina] mice but its lack results in the successful induction of immune regulatory pathways driven by myeloid cells that control CD8+ T cells resulting in protection from ECM.

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Combined IL17 and IL22 secretion profile characterizes the efficacy of MTB response

Y. Todorova1, R. Embilova1, V. Milanov1, M. Zamfirova1, T. Vatelova1, M. Nikolova1
1National Reference Laboratory of Immunology, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria; 2Clinic of Phthisiology, Multidrome Hospital for Active Treatment of Tuberculosis, Sofia, Bulgaria.

Tuberculosis remains a major cause of death and morbidity worldwide. Current interferon-γ release assays for diagnosis of MTB infection do not predict its clinical course and the need of specific therapy. The mechanisms of protective MTB-specific immune response have not been clarified. IL-17 and IL-22 were pointed out as key players, but data from human studies remain contradictory. Aim: To characterize IL-17 and IL-22 secretion profiles in subjects with different efficacy of MTB-specific immune response. Materials and methods: Peripheral blood samples from: A: Group I – healthcare workers in intensive contact with MTB (n=11); B: Group II – healthy persons (LTBI, n=21). IL-17 and IL-22 production was determined after 18h stimulation with phytohemagglutinin (A and B) or CD4+ and CD8+ specific MTB peptides (Quiagen) (B,C) by ELISA (cytokine, biotscience). Results: Secretion of IL-17 was significantly increased level of CD8 IL-22+ MTB-specific CD8 (162 vs. 77, p<0.05). Our data suggest that while both IL-17 and IL-22-CD8 T are engaged in the protective response to MTB, the level of IL-22+CD8 may be determinant for containment of latent infection. Supported by research grant No 13-1/14.12.2017, Bulgarian National Science Fund.

Effect of probiotics on cytokines gene expression in gingival epithelial cells challenged with Porphyromonas gingivalis

G. C. Vale1, E. A. Sando-Suguimoto1, E. Albuquerque-Souza1, M. P. Mayer1
1University of São Paulo, São Paulo, Brazil; 2Federal University of Piauí, Teresina, Brazil.

Although there is a vast amount of data supporting the pivotal role for cytokines in mediating the host response to periodontal pathogens and the associated tissue damage, literature is scarce regarding the mechanisms underlying the beneficial effect of probiotics. Thus, this study aimed to evaluate the effect of probiotics on gene expression of cytokines by gingival epithelial cells (GECs) challenged with Porphyromonas gingivalis. OBA-9 GECs (~2.5x 10^5 cells/well) were challenged with P. gingivalis strain (W83 or ATCC 33277) and co-infected with two tested probiotic strains (L. rhamnosus and L. acidophilus) at a multiplicity of infection (MOI) of 1:1,000 for 2h. OBA-9 viability was measured by trypan blue exclusion assay. Levels of gene expression encoding cytokines (IL-1β, IL-8, IL-6, IL-18, TNFa) were evaluated by RT-qPCR. P. gingivalis challenge with both strains resulted in a significant decrease of OBA-9 cells viability, which was partially reversed by the use of both probiotics. P. gingivalis W83 or ATCC 32377 promoted an increase in the transcription of pro-IL-1β, IL-8, and TNFa when compared to control no infected cells (OBA). The addition of both probiotics to P. gingivalis challenged cells resulted in decreased production of IL-1β and TNFa. Furthermore, challenged OBA-9 cells showed decreased expression of IL-18, which was further decreased by co-infection with the probiotics. Overall, the two probiotics have induced an altered cytokines expression profile, regarding the transcription of other inflammatory mediators such as IL-6 and IL-8. In conclusion, the probiotics tested boosted the human immunomodulatory effect on gingival epithelial cells.
Materials and Methods: Serum and M.tb-stimulated whole blood cultures were obtained from 95 adult individuals with active pulmonary TB classified into two groups according to the extent and type of chest radiograph findings. The studied proteins in sera and plasma were determined immunosynthetically (Dudicet5, ELISA, R&D). Results: The results showed the similar levels of IL-18, IL-37 and IP-10 in patients with severe and non-severe forms of TB. Significantly higher levels of IL-18BP in M.tb-stimulated cultures of blood of severe TB cases compared with non-severed patients were noticed (p<0.04). The levels of IL-18BP were correlated with the concentration of IL-18 in such cultures. Severe TB cases were characterized by increased ratio of IL-18BP/IL-37 and IL-18/IL-37 in serum, as well as IL-18BP/IL-37 and IL18BP/free IL-18 in M.tb-stimulated cultures. Conclusion: The ratio of IL18BP/IL-37 measured in both serum and M.tb-stimulated cultures may serve for distinguishing severe TB forms from non-severe. This work was supported by the National Science Centre Grant no. 2015/19/N/NZ6/01385 and 2016/21/B/NZ7/01771.

P.D4.04 Exploiting host pathogen interaction - Part 4

P.D4.04.01 The translation elongation factor-1 alpha (Tef1) of Candida albicans modulates mouse CD4+ T cell responses in vitro K. Alberter1, P. Dosari2, P. Zipse1, N. Beyerstorff2; 1University of Würzburg, Institute of Virology and Immunobiology, Würzburg, Germany, 2Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Jena, Germany. Invasive infections with the saprophytic yeast Candida albicans are a major cause of morbidity in immunocompromised patients. While the interaction of cells and molecules of innate immunity with C. albicans has been studied to great depth, comparatively little is known about the modulation of adaptive immunity by C. albicans. In particular, direct interactions of proteins secreted by C. albicans with CD4 T cells has not been studied extensively. We here report that the translation elongation factor-1 alpha (Tef1), which is secreted by C. albicans, binds to mouse CD4+ T cells and to a lower degree also to mouse B and CD8+ T cells. Functionally, purified Tef1 recombinantly expressed in Pichia pastoris enhanced IFNγ and IL-17 secretion by anti-CD3 monoclonal antibody-stimulated splenocytes identifying it as a factor inducing pro-inflammatory cytokine secretion. Our preliminary data further indicate that CD4+ Foxp3+ CD25+ regulatory T cells (Treg) bind Tef1 much better than CD4+ Foxp3+ CD25+ conventional CD4+ T cells. We assume that binding of Tef1 to Treg might ‘neutralize’ its activity as IFNγ secretion upon anti-CD3 monoclonal antibody and Tef1 stimulation was enhanced in the absence of Treg. The interaction of Tef1 and Treg might, thus, contribute to the commensalism of C. albicans and might be an important pathway protecting the organism from overshooting pro-inflammatory immune responses in invasive or even septic candidiasis. This study was funded by a grant from the DFG (CRC124 FungNet - project C6).

P.D4.04.02 An impact of holo-transferrin on release of Neutrophils Extracellular Traps (NETs)

W. Bystrzycka1, A. Mando-Handzik1, M. Wachowska1, U. Demkow1, O. Ciepielea; 1Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Warsaw, Poland, 2Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland. Metal ions, were reported to be found in the structure of Neutrophils Extracellular Traps (NETs). However, to this end, little is known about the exact functions of these ions in NETs formation. Taking into account the invaluable role of microelements in innate immunity, an in-depth understanding of the impact of metal ions on NETs release is desirable. The aim of this study was to investigate the impact of human holo-transferrin (Ht) on the release of NETs. Neutrophils were isolated from the blood of healthy donors using density gradient centrifugation method and incubated with Ht. PMA or calcium ionophore C2 were added to stimulate NETs release. The process of NETs release was assessed by post stimulation by fluorescent microscopy and fluorometry. Intracellular production of reactive oxygen species (ROS) was assessed by fluorimetrical analysis using dihydrorhodamine 123 (DHR123). Nitro blue tetrazolium (NBT) reduction assay was performed to determine how much superoxide is produced. The delivery of Ht to neutrophils contributed to an inhibition of netosis after stimulation with PMA. Transferrin at concentration of 5 μg/ml significantly decreased the amount of reactive oxygen species (ROS). Analysis of oxidative burst by DHR oxidation and NBT reduction assay exposed that Ht does not affect ROS release. Moreover, Ht did not affect NAPDH-oxidase-independent NETs release. Iron is a molecule involved in the formation of NETs. Further studies focusing on the mechanism in which Ht affects netosis are of great importance. This study was supported by funding from the National Science Centre, Poland; Preludium grant no. 2017/25/N/NZ6/00142 (WB).

P.D4.04.03 Antimicrobial peptide derived from chemokinetic factor and adipokine - chemerin provides protection against skin invading bacteria by targeting bacteria inner membrane J. Cichy1, U. Godlewska2, A. Zegar1, B. Bliska3, P. Kuleta4, E. Pysz3, A. Oszczczu3, B. A. Zaber3; 1Faculty of Biochem. Biophys. & Biotech. Jagiellonian Univ., Krakow, Poland, 2Institute of Zoology and Biomedical Resarch, Jagiellonian Univ., Krakow, Poland, 3Polo Alto Veterans Institute for Research, Palo Alto, United States. Antimicrobial peptides originating from endogenous human proteins have received significant attention as potential drug targets. Chemerin is chemoattractant and adipokine. Given abundance of chemerin in epidermis and subcutaneous fat tissue, chemerin and chemerin-derived peptides may confer protection against skin invading microbes. Therefore, understanding the modes of action of peptide 4 (p4), the most potent antimicrobial chemerin derivative is of high significance. Here we demonstrate that p4 binding to bacteria and its bactericidal activity were critically dependent on formation of disulfide-stabilized dimers, suggesting that p4 acts as antimicrobial agent under oxidized conditions. High doses of p4 rapidly damaged internal membrane of bacteria but did not cause lysis of human erythrocytes. P4 in either lethal or sublethal concentration was found to interfere with bacteria respiratory chain function by inhibiting cytochrome bc1-dependent pathway. These data provide new insights into how chemerin shapes host defense by showing previously uncharacterized mechanisms of antimicrobial activity of chemerin derived peptide. This work was supported by Polish National Science Center grant UMO 2014/12/W/NZ6/00454. The Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University is a partner of the Leading National Research Center (KNOW) supported by the Polish Ministry of Science and Higher Education.

P.D4.04.04 In vitro and in vivo posology optimization for an original bacterial immunomodulator

S. I. Ciulean; 1Cantacuzino’ National Institute for Medical-Military Research and Development, Bucharest, Romania. This study aims to evaluate different oral formulations for an original 13 strain, heat inactivated, bile-lysed, gram positive and negative bacterial immunomodulator formerly used as an injectable product. THP1 differentiated monocytes were exposed to stimuli derived from the immunomodulator’s composition.TLR4 blockage effects were determined on similar cultures. Female BALB/c mice were administered the immunomodulator in food (2ml/day) or water (10%) for 5 days. Cytokines, NFκB and intracellular reactive oxygen species (ROS) were determined in the spleen, bone marrow and Peyer patches and in THP1 culture supernatants. Animal experiments were performed by the Internal Ethics Committee (CE/101/24.06.2016). Gram negative bacteria determined higher levels of NFκB and proinflammatory cytokines compared to gram positive. An immunomodulator stimuli equivalent induced greater TNFα and IL8 production compared to the control and stimuli equivalent. Oral administration did not alter animal health. Compared to the control, both immunomodulation did not alter animal regulated serum MMP9 levels, tended to increase IL-10 secretion in Peyer patches and to have a proinflammatory effect in water compared to food administration. Bile-lysed immunomodulator water formulation determined high NFκB levels in Peyer patches. Food administration induced a lower medullar ROS production and inconsequential changes in the cytokine profile of culture supernatants from investigated organs. Our data suggests that both immunomodulatory formulations have pro-inflammatory effects in vitro and anti-inflammatory properties in vivo, probably due to oral administration. This study was supported by PN16390207 project granted by The Ministry of Research of.
Introduction: Tuberculosis (TB) as a consequence of Mycobacterium tuberculosis (M.tb) infection results from breaking the balance between protective immunity and destructive pathology. Aim: Since the correlates of protective immunity against TB are not known, our study focused on cytokine profiles of sera and soluble effectors of blood cells responding to M.tb antigens and aimed at the identification of immunological signatures of protective immunity and M.tb-induced pathology in childhood TB. Materials and Methods: In total, 163 BCG-vaccinated HIV-negative pediatric patients were investigated. All children underwent standard clinical and radiological examination including the interferon-gamma release assay (IGRA) testing. On the basis of the results of the current diagnostics children were classified into three groups: TB children, IGRA(+) and IGRA(-) children. A 15-plex Human Th1 Panel (Bio-Rad) was used to measure the concentration of IL-17 pathway-related cytokines in serum and plasma samples recovered from whole blood cultures stimulated with M.tb-specific antigens performed during IGRA testing. Results: The quantification of 16 proinflammatory and regulatory cytokines and chemokines showed that serum levels of IL-4, IL-10, IL-21 and IL-22 were significantly higher in TB patient group than IGRA(+) children. Moreover, the concentration of IP-10 in M.tb-stimulated cultures of blood from TB children was significantly increased as compared to IGRA(+) and IGRA(-) pediatric patients. Conclusion: The analysis of serum/whole blood cultures cytokine profiles may be a useful correlate of active TB in children. This work was supported by the National Science Centre Grant no. 2016/21/B/NZ7/01771.

POSTER PRESENTATIONS

P.D4.04.06
IL-10 overexpression during the initial steps of infection mediates susceptibility to Leishmania donovani infection
I. Mesquita,1 A. F. Ferreira,1 A. M. Barbosa,1 C. M. Ferreira,1 D. Moreira,1 A. Carvalho,1 C. Cunha,1 F. Rodrigues,1 N. D. Oliveira,1 I. Estaiuqi,1 A. Castro,1 E. Torrado1, S. Silvestre1
1Life and Health Sciences Research Institute (ICVS), Braga, Portugal, 2ICVS-38’s – PT Government Associate Laboratory, Braga/Guimarães, Portugal, 3INFACTS – Institute of Research and Advanced Training in Health Sciences and Technologies, Department of Sciences, University Institute of Health Sciences (IUSCS), CESPU, CRG, Gandara, Portugal, 4UCIBIO, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal, 5Department of Public Health and Forensic Sciences, Faculty of Medicine, University of Porto, Porto, Portugal, 6CNRNS FR 3636, Université Paris Descartes, Paris, France, 7Centre de Recherche du CHU de Québec, Université Laval, Quebec G1V 4G2, Canada.

Leishmaniasis is a vector-borne disease caused by protozoan parasites from the genus Leishmania. The most severe form of disease is visceral leishmaniasis (VL), which is fatal if left untreated. It has been demonstrated that interleukin (IL)-10, a potent anti-inflammatory cytokine, is associated with disease progression and susceptibility. We took advantage of a transgenic mouse model that expresses high levels of IL-10 upon zoite (pMT-10) infection. We addressed the role of IL-10 during the initial stages of L. donovani infection by analyzing the parasite burden in the spleen and liver of the infected pMT-10 and WT mice as well as the histopathological alterations upon IL-10 induction. Furthermore, the profile of cytokines expressed by T cells was assessed.

Our results demonstrate that high levels of IL-10 during the initial 12 days of infection leads to higher susceptibility to VL, demonstrated by increased parasite burden in the spleens and livers of infected pMT-10 animals, compared to WT controls. Further analysis revealed that the increased susceptibility of pMT-10 animals was also associated with increased levels of alarmins transaminase and aspartate transaminase, usually related with hepatic toxicity. Interestingly, the observed phenotype is also correlated with a decreased frequency of multifunctional CD4 T cells and decreased IFN-γ/IL-10 ratio, further associated with susceptibility with Leishmania infection. Such immunologic landscape contributes for the establishment of a successful infection.

Our data suggests that the overexpression of IL-10 during the initial steps of the infection impacts host ability to control L. donovani infection by limiting the development of a protective adaptive immune response.

P.D4.04.07
Human dendritic cell sequestration onto the Necator americanus larval sheath through ex-sheathing: a possible mechanism for immune privilege
A. Hassan1, A. M. Ghaseimmaghami1, D. I. Pritchard1, 1Division of Immunology, School of Life Sciences, Faculty of Medicine & Health Sciences, University of Nottingham, Nottingham, United Kingdom, 2School of Pharmacy, University of Nottingham, Nottingham, United Kingdom.

Despite the profound health implications of Necator americanus infection in humans, many aspects of its interaction with the host immune system are poorly understood. Here we investigated the early events at the interface of N. americanus larva (L3) and human dendritic cells (DCs). Our data show that co-culturing DCs and the larvae trigger ex-sheathing of hookworms rapidly where a majority of DCs are sequestered onto the larval sheath allowing the ex-sheathed larvae to migrate away unchallenged. Intriguingly, DCs show negligible interaction with the ex-sheathed larva, alluding to differences between the surface chemistry of the larva and its sheath. Furthermore, blocking of two key C-type lectin receptors on DC surface (i.e. DC-SIGN and mannose receptor) resulted in inhibition of ex-sheathing process and DC sequestration, highlighting the importance of C-type lectins on DCs in the induction of the ex-sheathing. Analyses of DC phenotype and cytokine profile after co-cultures with the N. americanus larvae showed an immature phenotype whereas the larvae expressed IL-10 and IL-18, promoting pyroptosis. Here, we characterise Glutathione transferase omega 1-1 (GSTO1-1) as a critical NLRP3 inflammasome regulator. Using a small molecule inhibitor of GSTO1-1 termed C1-27, endogenous GSTO1-1 knockdown and GSTO1-1/-/- mice, we report that GSTO1-1 is required for NLRP3 inflammasome activation.

Mechanistically, GSTO1-1 de-glutathionylates 253 in NIMA related kinase 7 (NEK7) to drive NLRP3 activation. This is the first report of GSTO1-1 as an NLRP3 inflammasome component, and also identify GSTO1-1 as a drug target to limit NLRP3 inflammasome-mediated inflammation.

P.D4.04.09
Identification of the first naturally processed CD4+ T cell epitope of mumps virus
P. Koppel1, M. E. Emmelot, M. C. Poelen, W. Han, C. A. van Els, J. de Wit, 1IVM, Bilthoven, Netherlands.

Several mumps outbreaks have been reported among young adults despite vaccination. Poor induction of the T cell response after vaccination may play a role, but has not yet been studied extensively. T cell epitopes can be useful in exploring the host immune response to mumps virus in more detail, but so far no epitope has been identified. Mumps virus nucleocapsid may be a good target for T cell responses as it has been identified as immunodominant protein, and other viral nucleoproteins have already shown to be a major T cell target. A CD4+ T cell clone was generated from a mumps case using recombinant mumps nucleoprotein as antigen. The T cell clone proved to be directed against a naturally processed epitope, as it recognized mumps virus-infected cells using a 2D matrix peptide pool of 15-mers peptides covering the complete protein, the peptide-specificity could be identified as GTRYPARVANILA. Upon peptide-specific stimulation, the T cell clone responded in a HLA-DR restricted manner by expression of the activation marker CD137, indicating the potential of this T cell clone for prophylactic use.

P.D4.04.10
Glycan-mediated binding of extracellular vesicles from Schistosoma mansoni juvenile worms to DC-SIGN on dendritic cells triggers cytokine release
1LUMC, Leiden, Netherlands, 2Utrecht University, Utrecht, Netherlands, 3Aberystwyth University, Aberystwyth, United Kingdom.

Extraacellular vesicles (EV) are known intercellular communicators and can transport various molecular cargo. Although it is known that the parasitic worm Schistosoma mansoni releases EV, their exact composition and interaction with the host immune system are largely uncharacterised. One of the main classes of molecules from schistosomes involved in parasite-host interaction are glycans. Therefore, we investigated the glycolocalization of EV released by S. mansoni juvenile worm (schistosomula) and their effect on human monocyte-derived dendritic cells (moDC), known to be key cells affected by schistosome-derived products.
A. Obraztsov, I. Shaidova, and D. Paramonov

**Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands**
Infectious disease causes respiratory disease leading to 500,000 deaths each year worldwide. During infection, one important component of the innate immune response is the expression of type I interferons (IFN-I). Signalling through its receptor IFNAR1/IFNAR2, this cytokine family will interfere with viral replication and induce an antiviral state in infected and bystander cells. Many publications demonstrated the beneficial effects of a moderate IFN-I response during infection. However, in humans, severe influenza is associated with high IFN-I levels. Similarly, mouse strain 129 responds to influenza infection by producing excessive IFN-I amounts that drive pathogenic innate immune responses. Genetic removal of IFN-I signalling ameliorates disease. Depletion experiments determined that IFNα was mainly produced by plasmacytoid dendritic cells (pDCs). The signal inducing pDCs to produce IFNα in influenza infection is commonly thought to be Toll-like receptor 7 (TLR7)-mediated recognition of viral RNAs. While Ab-mediated pDC depletion ameliorated disease in severe influenza in 129 mice, a pharmacological approach using an influenza-triggered TLR7 663 was tested in vitro and showed a high efficiency at reducing influenza-triggered IFNα production by bone marrow-derived pDC already at 0.75μM. In vivo, 661 anti-IFNα effects during influenza infection were observed only when administered locally (intranasally) at a dose of 4.8μg/kg. Studies are currently ongoing to determine if reducing excessive IFNα production during influenza infection in the pro-inflammatory 129 model will reduce overall influenza severity.

Candida albicans CRASP11 modulates the host-mediated dendritic cell response by binding the pattern-recognition-receptors dectin-1 and dectin-2

C. J. Stainker\(^1\), M. Meurs\(^2\), Y. Mueller\(^1\), J. Brouwers-Haspels\(^1\), S. Erkeland\(^1\), P. Lohning\(^2\)
\(^1\)Erasmus University Medical Center, Rotterdam, Netherlands, \(^2\)Drexel University College of Medicine, Philadelphia, United States.

Influenza virus infection poses a serious threat to public health. It infects lung alveolar epithelial cells in the respiratory tract, utilizing them to reproduce, spreading the infection. Understanding genes that are dynamically regulated during influenza virus infection may help elucidate essential genes and pathways that affect influenza virus infection. We examined the in vivo RNASeq gene expression profiles of alveolar epithelial cells sorted from A/Puerto Rico/8/1934-GFP (PR8-GFP) expressing influenza virus infected mice. We identified a novel influenza virus-induced gene, Heatr9 (also known as Gm14435), that was upregulated >200-fold in vivo in mouse alveolar epithelial cells. The upregulation of Heatr9 by influenza virus infection was further confirmed in vitro in PR8-GFP influenza virus infected human lung A549 cells. In vivo Heatr9 upregulation was found to be indirect as bystander alveolar epithelial cells in lungs exhibited similar levels of Heatr9 induction as infected cells. Furthermore, supernatants of influenza virus infected A549 cells were capable of potently inducing Heatr9 mRNA even in the absence of infection. To identify factors that upregulate Heatr9 we examined the effect of cytokines on Heatr9 expression in vitro. Although not induced by IFNα, TNFα, and IL-1β alone in A549 cell, when used in combination, IFNα and TNFα or IFNα and IL-1β potently induced Heatr9 mRNA. Currently, we are generating Heatr9 deficient cell lines to examine the function of this gene in infection. In summary, we have identified Heatr9 as a cytokine- and influenza virus infection inducible gene, the function of which has yet to be determined.

Using oligonucleotides to control a dysregulated type I interferon response in severe influenza

J. C. F. Rappe, A. Wack;
Francis Crick Institute, London, United Kingdom.

Influenza virus infection causes respiratory disease leading to 500,000 deaths each year worldwide. During infection, one important component of the innate immune response is the expression of type I interferons (IFN-I). Signalling through its receptor IFNAR1/IFNAR2, this cytokine family will interfere with viral replication and induce an antiviral state in infected and bystander cells. Many publications demonstrated the beneficial effects of a moderate IFN-I response during infection. However, in humans, severe influenza is associated with high IFN-I levels. Similarly, mouse strain 129 responds to influenza infection by producing excessive IFN-I amounts that drive pathogenic innate immune responses. Genetic removal of IFN-I signalling ameliorates disease. Depletion experiments determined that IFNα was mainly produced by plasmacytoid dendritic cells (pDCs). The signal inducing pDCs to produce IFNα in influenza infection is commonly thought to be Toll-like receptor 7 (TLR7)-mediated recognition of viral RNAs. While Ab-mediated pDC depletion ameliorated disease in severe influenza in 129 mice, a pharmacological approach using an influenza-triggered TLR7 663 was tested in vitro and showed a high efficiency at reducing influenza-triggered IFNα production by bone marrow-derived pDC already at 0.75μM. In vivo, 661 anti-IFNα effects during influenza infection were observed only when administered locally (intranasally) at a dose of 4.8μg/kg. Studies are currently ongoing to determine if reducing excessive IFNα production during influenza infection in the pro-inflammatory 129 model will reduce overall influenza severity.

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Candida albicans CRASP11 modulates the host-mediated dendritic cell response by binding the pattern-recognition-receptors dectin-1 and dectin-2

N. Reither\(^1\), M. Reza\(^2\), C. Skerka\(^2\), P. Ziegel\(^1\),
\(^1\)Center for Sepsis Control and Care, Jena, Germany, \(^2\)HKI-Leipzizn Institute for Natural Product Research and Infection Biology, Jena, Germany.

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J. C. F. Rappe, A. Wack;
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Influenza virus infection causes respiratory disease leading to 500,000 deaths each year worldwide. During infection, one important component of the innate immune response is the expression of type I interferons (IFN-I). Signalling through its receptor IFNAR1/IFNAR2, this cytokine family will interfere with viral replication and induce an antiviral state in infected and bystander cells. Many publications demonstrated the beneficial effects of a moderate IFN-I response during infection. However, in humans, severe influenza is associated with high IFN-I levels. Similarly, mouse strain 129 responds to influenza infection by producing excessive IFN-I amounts that drive pathogenic innate immune responses. Genetic removal of IFN-I signalling ameliorates disease. Depletion experiments determined that IFNα was mainly produced by plasmacytoid dendritic cells (pDCs). The signal inducing pDCs to produce IFNα in influenza infection is commonly thought to be Toll-like receptor 7 (TLR7)-mediated recognition of viral RNAs. While Ab-mediated pDC depletion ameliorated disease in severe influenza in 129 mice, a pharmacological approach using an influenza-triggered TLR7 663 was tested in vitro and showed a high efficiency at reducing influenza-triggered IFNα production by bone marrow-derived pDC already at 0.75μM. In vivo, 661 anti-IFNα effects during influenza infection were observed only when administered locally (intranasally) at a dose of 4.8μg/kg. Studies are currently ongoing to determine if reducing excessive IFNα production during influenza infection in the pro-inflammatory 129 model will reduce overall influenza severity.
P.D4.05.03
Characterisation of interferon-induced protein 44 mediated inhibition of respiratory syncytial virus (RSV) infection
D. C. Buse1, S. Smith1, C. Brandt1, S. Clare1, P. Kellami1, J. S. Tregonning1
1Imperial College London, London, United Kingdom. 2Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

RSV infection represents a major global cause of morbidity and mortality, yet the factors that influence disease severity are not fully understood. One possibility is that disease severity is linked to the ability of the infected host cells to recognise and control viral infection. Respiratory viral infection triggered the production of interferons which in turn induce an array of intracellular antiviral proteins encoded by interferon-stimulated genes (ISGs). Whilst some ISGs have been linked to the development of severe RSV disease, little is known about the mechanism of many of these important antiviral genes. The ISGs IFN-Induced protein 44 (IFI44) and IFN-induced protein 44-like (IFI44L) are upregulated after RSV infection, but they do not as yet have a defined mechanism of action. We hypothesised that their upregulation after infection was linked to a protective role. The effects of IFI44 and IFI44L on viral infection were analysed via siRNA-mediated knockdown or through overexpression in relevant human cell lines. The impact of IFI44 on RSV infection was further investigated in IFI44- mice. Both IFI44 and IFI44L were confirmed as ISGs upregulated during RSV infection both in vitro and in vivo. Knockdown of IFI44 in epithelial cell lines resulted in elevated viral infection. RSV infection in IFI44- mice was associated with more severe disease relative to wild type controls with increased viral load during infection. This therefore suggests that IFI44 plays an important antiviral role in the prevention of RSV infection.

P.D4.05.04
Beta-glucan modulates human macrophage differentiation and polarization toward macrophages with unique properties
G. Camilli, J. Quintin;
Posteur Institute, Paris, France.

Beta-glucan, a naturally derived polysaccharide present in the cell wall of fungi, positively impact the outcome of cancer and a number of infectious diseases, although the exact mechanism remain to be elucidated. Macrophages derived from monocyte precursors undergo specific differentiation that depends on microenvironmetal cues. In tissue, they mature and acquire distinct functional phenotype in response to environmental cues. Evidences suggest that beta-glucan treatment converts the immunosuppressive M2 (alternatively activated macrophages) and TAM (tumor associated macrophages) toward the M1 (classically activated macrophages) pro-inflammatory and antitumor phenotype. Recently, several seminal studies showed that stimulation of human monocytes by beta-glucan bring monocytes into a long-term enhanced functional state, through metabolic and epigenetic changes (trained immunity). Trained monocytes show a stronger proinflammatory response to a second stimulus and provide non-specific protection against several types of infections. Although a stronger proinflammatory response is generally acknowledged as a mechanism of controlling infection and cancer progression, it can in some circumstances cause damage to healthy tissue and contribute to pathology. As such, exacerbated secretion of inflammasome-derived cytokines (i.e. IL-1β) have a critical pathogenic role in several inflammatory diseases.

Providing the new concept of trained immunity and its non-specific protective properties, we sought to investigate how the fungal beta-glucan modulates the differentiation of human monocytes into macrophages. Here, we show that treatment of human monocytes with fungal beta-glucan skews their differentiation, triggered by either M1 or M2 stimuli, into macrophages with a specialized functional phenotype. As such, these macrophages exhibit a non-deleterious secretory profile of inflammasome-related cytokines. In this setting, we propose that beta-glucan treatment induces a non-deleterious specific functional phenotype that we called beta-glucan-induced trained macrophages (beta- TMs).

P.D4.05.05
Role of Immunoglobulin A in Mycoplasma pneumoniae upper respiratory tract carriage
R. C. A. de Groot, P. M. Meyer Sautere1, L. M. Verhagen1, E. B. Spuesens3, S. E. Estevo1, T. Hoogenboezem1, L. M. Rossum1, W. W. Unger1; 1Erasmus MC, Rotterdam, Netherlands, 2University Children's Hospital of Zurich, Zurich, Switzerland, 3Utrecht University Medical Center – Wilhelmina Children's Hospital, Utrecht, Netherlands.

Mycoplasma pneumoniae (MP) is the most common bacterial cause of community-acquired pneumonia in children. Infection in the upper respiratory tract (URT) is preceded by asymptomatic carriage in the upper respiratory tract (URT). We studied the role of the humoral response to MP in the URT and compared it to the LRT.

Methods: MP or medium was installed intranasally in C57BL/6 or C3H-10T1/2 mice. Healthy children and children with selective IgA deficiency (sIgAD) were recruited. On respiratory tract samples we determined MP copy number by qPCR and MP-specific IgA, IgM and IgG titers using an in-house ELISA.

Results: In MP infected mice, MP-specific IgG was markedly elevated in the bronchoalveolar lavage fluid. In contrast, the nasal lavage fluid contained high levels of MP-specific IgA. Serum transfer of infected wild-type mice to C3H-10T1/2 mice rescued URT clearance of MP. Interestingly, the serum transfer had no effect on MP load and MP-specific IgA levels in URT. Immunofluorescence showed the presence of positive IgA cells in the URT starting from day 7. To translate our findings to children we measured MP-specific antibodies in children. We included 33 children with sIgAD and 477 healthy control subjects.

Conclusions: MP-specific IgG responses dominated in the URT, whereas MP-specific IgA was increased in the LRT, where it seemed to lower MP load. Insights into the humoral response to MP can benefit vaccine development and immunoglobulin treatment of patients with primary antibody deficiency.

Acknowledgement: This research was supported by Sophia Research Foundation (grant S18-04 to RGG)

P.D4.05.06
The effect of immunonutrient intake on severity of pulmonary tuberculosis clinical symptoms
T. Faadhila, C. Brandi, A. Anu, I. M. Sari; Andalas University, Padang, Indonesia.

Introduction: Tuberculosis (TB) is one of the top 10 causes of death worldwide. The severity of pulmonary TB symptoms is affected by the immune status which is strongly influenced by nutrition. Immunonutrient can be defined as the effect of the provision of specific nutrients on immune function.

Materials and methods: The method of this study is cross sectional study of 52 pulmonary tuberculosis patients. They were interviewed with questionnaire of FFQ. The amount of immunonutrient on severity of pulmonary tuberculosis clinical symptoms.

Results: The average intake of vitamin D and protein intake is calculated by using the nutrisurvey. Clinical symptoms of TB patients are measured by using Bandim TBScore. Statistical analysis of the two variables is allegedly linked using SPSS.

Conclusions: Based on the statistical analysis, vitamin D and protein intake are not significantly affect the severity of pulmonary TB clinical symptoms. However, the average intake of vitamin D is 92.3% (48 persons), TBScore II is 7.69% (4 persons), TBScore III is 0% (0 persons). The average of TBScore is 2.04 ± 1.89.

Acknowledgement: The effect vitamin D and protein intake is calculated by using the nutrisurvey.

P.D4.05.07
Immunomodulatory role of radiations in the control of Epstein-Barr virus fate
D. Falichia1, C. Procaccini1, S. Brizzuratti1, C. Fusco1, T. Michiò1, M. Crane1, A. Faggioni1, G. Mattarese1; 1Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche, Napoli, Italy, 2Dipartimento di Medicina molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Naples, Italy, 3Dipartimento di Biologia, Università di Napoli Federico II, Naples, Italy, 4Department of Experimental Medicine, “Sapienza” University of Rome, Roma, Italy.

Autophagy is a catabolic pathway involved in cell survival under stress conditions. Cells engage autophagy as a detoxification mechanism and/or to solve infections, but some microorganisms are learning to evade it or to appropriate of this machinery for their own benefit. Recent studies have highlighted a central role of autophagy in supporting the lytic phase of Epstein-Barr virus (EBV). Furthermore, data from literature have suggested a potential immunomodulatory role exerted by ionizing radiation in controlling viral fate. In this study, we investigated the effect of radiations on immortalized B lymphocytes, which have integrated EBV in epigenomic. Upon radiation exposure, EBV-infected B cells showed a reduced activation of the mammalian-target of rapamycin (mTOR) pathway, which resulted in an increased autophagic flux. Concomitantly, in EBV-infected B cells, irradiation induced the expression of proteins of the early phases of EBV lytic cycle (Zebra, EA); these phenomena were accompanied by an enhanced release of pro-inflammatory cytokines (IFN-γ, IL-17, TNF-α), a reduced secretion of anti-inflammatory cytokines (such as IL-10), together with an increased expression of the activation marker CD40 on infected B cells. Taken together, these data suggest a key role of radiation in EBV latency in lymphocytes and in the reactivation of viral lytic cycle, through autophagy induction. A better understanding of the mechanisms that regulate the interplay between radiation and viral activation could help to improve the treatment of EBV-associated diseases.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

**POSTER PRESENTATIONS**

**P.D.04.05.08**

**Listeria monocytogenes** adapt to the host cells by inducing the AP-1/Fra-1 level thus inhibiting the guanylate-binding proteins expression

A. Hännemann, S. Coz, G. Schett, D. Soutsil, A. Boeck;

1. Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Department of Internal Medicine 3 – Rheumatology and Immunology, Universitätsklinikum Erlangen, Erlangen, Germany;

2. Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Institute of Microbiology Clinical Microbiology, Immunology and Hygiene, Universitätsklinikum Erlangen, Erlangen, Germany.

Macrophages have a broad arsenal of microbialic features. During Listeria monocytogenes (L.m.) infection, they are the first line of defence against liver and spleen damage. Listeria’s transporter (AP-1) transcription factor family, specifically its subfamily of FOS proteins (cFos, FoDl, Fra-1 and Fra-2), can regulate macrophage cytokines production. To delineate whether Fra-1 is involved in the antimicrobial defence of macrophages, chromatin-immunoprecipitation (CHIP) sequencing analysis using thio-glucoyl-caceted macrophages pooled down for Fra-1 was performed. This analysis revealed that Fra-1 binds to the promoter regions of guanylate-binding protein (Gbp) -11, -2b, 3 and 5. To investigate whether Fra-1 is involved in the defence against L.m. infection, bone marrow-derived macrophages (BMDM) from Fra-1 deficient and wildtype mice were infected with L.m. (MDQ10). Interestingly, we found a decreased CFU in Fra-1 deficient BMDM. In accordance decreased LDI levels reflecting a decreased toxicity was quantified in the supernatant of the Fra-1 deficient cells when compared to controls. RNA analysis of infected BMDM revealed no difference in type-1 interferons, but increased expression of Gbp-5, -6 and -10. To address the role of Fra-1 in vivo, Fra-1 deficient mice (Fra-1<sup>−/−</sup>Mac<sup>Cre</sup>) and their wildtype littermates were infected intraperitoneally with L.m. In line with the previous finding, CFU of L.m. was reduced in the spleen and liver of Fra-1 deficient mice. Moreover, the expression of Gbps-5, -6, -7 and -10 was increased in the liver and spleen of Fra-1 deficient mice. Our data suggest that Fra-1 in macrophages inhibits microbial factors, especially GBP's, promoting bacterial growth.

**P.D.04.05.09**

**Sirtuin 5 deficiency does not compromise innate immune responses to bacterial infections**


1. Lausanne University Hospital, Epalinges, Switzerland, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

**Background and aim:** Sirtuins (SIRT1-7) belong to the highly conserved family of NAD<sup>+</sup>-dependent lysine deacetylases. SIRT5, one of the least characterized sirtuins, resides mainly in the mitochondria and catalyses lysine deacetylation, demalonylation, desuccinylation and deglutarylization to regulate metabolic and oxidative stress response pathways. Pharmacologic inhibitors of SIRT5 are under development for oncologic conditions. Nothing is known about the role of SIRT5 in innate immune responses. The aim of the study was to investigate whether SIRT5 deficiencies impact from healthy human tissues.

**Methods:** Mice were housed in SPF conditions. Thymic and splenic subpopulations were analyzed by flow cytometry. Bone marrow-derived macrophages and splenocytes were stimulated with TLR ligands, bacteria, exotoxins and polyclonal activators and analyzed for metabolic, cytokine and oxidative stress pathways. Mice were challenged i.p. with L.p. or E.coli, i.n. with K.pneumoniae or S.pneumoniae and i.v. with L.monocytogenes or S.aureus. Blood was collected to quantify cytokines and bacteria. Weight, severity score and survival were registered.

**Results:** SIRT5 deficiency did not affect innate immune cell development. SIRT5 deficiency increased oxidative phosphorylation over glycolysis in macrophages, but did not modulate cytokine production and proliferation by macrophages and splenocytes. SIRT5 deficiency had no impact on cytokine blood levels, bacteremia and survival rates in models of endotoxemia, pneumonia, pentaoxins, sepsis and splenocytic sepsis.

**Conclusions:** These data suggest that SIRT5 has no major impact on innate immune responses to bacterial infections and support the safety profile, in terms of susceptibility to infections, of SIRT5-directed therapies under development for oncologic diseases.

**P.D.04.10**

**Platelets and the regulation of tissue destruction in tuberculosis.**

D. E. Kirwan, A. M. Whittington, K. A. Fox, R. H. Gillman, K. A. Taylor, M. Emerson, J. S. Friedland;

1. Imperial College London, London, United Kingdom, Johns Hopkins University, Baltimore, United States.

**Introduction** Tuberculosis (TB) is characterised by inflammation and immune-mediated tissue damage by enzymes, particularly matrix metalloproteinases (MMPs). Platelets are involved in immune cell regulation, but their role in TB is poorly understood.

**Methods** Platelet-derived markers were measured in 50 TB patients before and during treatment, and in 50 healthy controls. Fresh platelets were incubated with live, virulent TB, TB-derived secreted antigens, or control medium for 30min. Monocytes were cultured with autologous platelets ±TB for 24h. Supernatants were analysed by ELISA and gene expression by qPCR. Platelet function was assessed using light transmission aggregometry.

**Results** Platelet factor 4 (PF4) concentrations were significantly higher in TB patients than healthy controls (median 1,129 [IQR 1,769] vs 462.5 [IQR 693] ng/ml, p<0.0001). PF4 concentrations transiently increased at treatment day 14 and normalised by day 60 (612.7 [IQR 1,806] ng/ml, p=0.073 vs controls). CD40L, PDGFB-B, and PTX-3 concentrations followed similar trends.

**Conclusion** Incubation with TB did not affect platelet PF4 secretion but soluble TB antigens increased PF4 secretion from 141.8 ±6.6 to 255.8 ±13.8 ng/ml (p<0.0017) and also increased inflammatory platelet responses. Platelets significantly increased MMP-1 and -10 secretion from TB-infected monocytes from 231.9 ±29.1 to 1,820 ±59.2 ng/ml (p<0.0017) and from 530.8 ±45.3 to 1,341 ±84.2 pg/ml (p=0.014) respectively. MMP-1 and -10 gene expression were similarly upregulated.

**Conclusion** Platelet activity is increased in TB patients, which is driven by TB-infected monocytes which may contribute to TB immunopathology. Platelets may constitute a potential therapeutic target for TB.

**P.D.04.11**

**Effector mechanisms of neutrophilic granulocytes in realization of systemic and local immune response in children suffering from purulent-inflammatory diseases of soft tissues**

I. V. Nesterova, G. A. Chudilov, V. A. Torokanov, N. K. Barov, T. B. Rusinova, S. V. Kovalcov, A. A. Agelevsky;

1. Peoples’ Friendship University of Russia, Moscow, Russian Federation, 2. Institute of Microbiology Clinical Microbiology, Immunology and Hygiene, Peoples’ Friendship University of Russia, Moscow, Russian Federation.

**Introduction** Neutrophilic granulocytes (NG) play a crucial role in antibacterial defense realizing its functions by phagocytose, transmembrane degranulation, formation of neutrophil extracellular traps (NET) and ectosomes. Staphylococcus (Stp.), causing the purulent-inflammatory diseases of soft tissues, emit virulence factors that violate the mechanisms of NG phagocytosis. We had studied 16 children (group 1), 3-8 years, suffering from purulent-inflammatory diseases (abscesses, phlegmons). Control group consist 7 healthy children. In both groups we evaluated NG phagocytosis. We had studied 16 children (group 1), 3-8 years, suffering from purulent-inflammatory diseases (abscesses, phlegmons). Control group consist 7 healthy children. In both groups we evaluated NG phagocytosis. We had studied 16 children (group 1), 3-8 years, suffering from purulent-inflammatory diseases (abscesses, phlegmons). Control group consist 7 healthy children. In both groups we evaluated NG phagocytosis.

**Methods** Fresh platelets were incubated with live, virulent TB, TB-derived secreted antigens, or control medium for 30min. Monocytes were cultured with autologous platelets ±TB for 24h. Supernatants were analysed by ELISA and gene expression by qPCR. Platelet function was assessed using light transmission aggregometry.

**Results** Platelets significantly increased MMP-1 and -10 secretion from TB-infected monocytes from 231.9 ±29.1 to 1,820 ±59.2 ng/ml (p<0.0017) and from 530.8 ±45.3 to 1,341 ±84.2 pg/ml (p=0.014) respectively. MMP-1 and -10 gene expression were similarly upregulated.

**Conclusion** Platelet activity is increased in TB patients, which is driven by TB-infected monocytes which may contribute to TB immunopathology. Platelets may constitute a potential therapeutic target for TB.

**P.D.04.12**

**Mitochondrial dynamic, beta tubulin and extracellular traps in cultures of human autologous leukocytes stimulated with LPS**

R. Rineru, M. V. Reyna, F. M. Rodriguez, I. Novak;

1. Institute of Cell Biology, Faculty of Medicine, Cordoba, Argentina.

**Introduction**: The endotoxemia produced by the effects of endotoxins such as LPS in the blood circulation lead to inflammation in multiple organs. Extracellular traps (ETs) are structures of extracellular and intracellular proteins which are extruded in leukocytes in inflammatory conditions. Some protein components of the cytoskeleton have been described in the traps, but the presence of beta-tubulin has not been reported. Not all ETs are created equal, this depend on source of stimulation. Mitochondria are currently considered to have regulatory functions of innate and adaptive immunity and are determinants in the phenotypes adopted by immune cells in their responses. Objectives: generation of ETs in leukocytes cultures, challenged with LPS and perform marking beta-tubulin and on the other hand, to observe the morphology characteristics of mitochondria of leukocytes in LPS assay. Methods: autologous leukocyte cultures from healthy human blood samples with ethical consent and anticoagulated blood were stimulated with 25 ng/ml ethidium monoazide methiodide (EMI) and 100 ng/ml of mito-tracker green. Immunofluorescence technique with anti-beta-tubulin Abs, DNA stain with DAPI. Paired blood samples provided controls. Cell pellets from cultures were performed to study with electron microscopy transmission. Results: beta-tubulin molecules were observed in ETs. We observed altered mitochondrial morphology in samples of LPS assay with an increase in size and sphericity with electronlucid images in leukocytes. Conclusions: the expression of beta-tubulin allow to better understand the composition of ETs generated by LPS. Is this similar in the ETs triggered by different stimulus? Mitochondrial morphological change induce to improve or deteriorate lymphocyte functions? Further experiments are necessary.
POSTER PRESENTATIONS

P.D4.05.13
CD72/CD100 and PD-1/PD-L1 markers are increased on T and B cells in HIV-1 viremic patients, and CD72/CD100 axis is correlated with T-cell exhaustion

During HIV-1 infection, PD-1/PD-L1 axis' role in dysfunction of the immune response was described, and high expression of PD-1 and PD-L1 was associated with an immunosuppression state by limiting HIV-1-specific T-cell response. On the other hand, CD100 was demonstrated to play a relevant role in immune response and its expression at the surface of T cells is unknown, although it may play a role in a low-deregulated manner during HIV-1 infection. We researched the PD-1/PDL-1, and CD72/CD100 axis-related markers expression on T and B cells in HIV-1 naïve-treated patients and in healthy individuals. We analyzed the frequencies and fluorescence intensities of these four markers on CD4+ and CD8+ T cells and on B cells. Expression of these markers was increased during active HIV-1 infection. The frequency of CD100 on T cells was positively associated with the expression of PD-1 and PD-L1 on T cells from naïve-treated HIV-1+ patients. In addition, the frequency of CD72/CD73 expressing T cells was associated to the IFN-γ production in naïve-treated HIV+ patients. Our data suggest that CD72/CD100 and PD-1/PD-L1 axes all together may participate in deregulation of immunity during HIV-1 infection and could explain in part the hyper-activation of the immune system.

P.D4.05.14
p.p.1 (margin: 0.09 0.0 0.0 0.0; font: 11.09 Arial) The long pentraxin PTX3 has a non-redundant role in the control of Streptococcus pneumoniae invasive infections
R. PORTE1, R. Parente1, M. Sirion1, F. Pasqualini2, T. van der Po1, C. Garlanda1, B. Bottazzi1, A. Mantovani1; 1Humanitas Clinical and Research Center, Pieve Emanuele (Milan), Italy, 2Academic Medical Centre, Amsterdam, Netherlands.

Pentraxin 3 (PTX3) is a fluid phase pattern recognition molecule which has served as a paradigm for linking the cellular and humoral arms of innate immunity. PTX3 is an important component of host resistance to pulmonary infections for selected pathogens. It was our aim to investigate the role of PTX3 in the control of pneumococcal infections caused by Streptococcus pneumoniae, the most common causative bacteria in community-acquired pneumonia and an important cause of mortality worldwide. By using a model of invasive pneumococcal infection in young adult mice, we observed a strong expression of PTX3 by non-hematopoietic cells. Comparing the pneumococcal load and survival of infected mice, we observed a higher sensitivity of PTX3+ animals during the invasive phase of the infection which could be restored by a systemic administration of recombinant PTX3. Infected PTX3− mice also showed an increased inflammatory profile. Furthermore, the local exogeneous instillation of PTX3 during the ongoing infection was able to reduce the expression of numerous inflammatory cytokines and the pneumococcal pulmonary load. We also observed that PTX3 specifically bind on S. pneumoniae but not in physiological condition in murine macrophages in vivo infection. The mechanism of the protective function of PTX3 remains to be fully elucidated. Our results suggest a non-redundant role of PTX3 in the control of S. pneumoniae infections. As inflammation and coagulation are important during pneumococcal invasive diseases, we are now studying the involvement of PTX3 in these systems for control of the infection.

P.D4.05.15
High incidence of primary immunodeficiencies in patients hospitalized for invasive pneumococcal diseases
E. Hernández-Brito1, E. Colino1, M. Garcia-Luzardo1, M. T. Martínez-Saavedra1, F. Artilés-Campeño1, M. Santana-Hernández1, N. Gonzalez-Quevedo1, C. Rodriguez-Gallego1; 1Department of Immunology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas, Spain, 2Department of Pediatrics, Hospital Universitario Materno-Infantil, Las Palmas de Gran Canaria, Las Palmas, Spain, 3Department of Microbiology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Las Palmas, Spain.

Introduction. Streptococcus pneumoniae is a leading cause of bacterial pneumonia, meningitis, and sepsis in children. Some primary immunodeficiencies (PIDs) confer predisposition to invasive pneumococcal disease (IPD). Methods. Identification of pediatric (younger than 14 years) patients with IPD between January 2000-February 2017 from the province of Las Palmas (one million inhabitants). Clinical and epidemiologic data and immunological explorations. Results. We identified 186 children who suffered from IPD, of whom 68 patients (mean age 32 months; range, 0 days-13 years) required hospitalization. Fourteen of the 68 children (20,6%) had classical risk factors. Immunological evaluation could be performed to 36 patients. Seven patients suffered from a genetically confirmed PID: IRAK-4 deficiency (1 patient), X-linked agammaglobulinemia (1), congenital asplenia (2), Ataxia-telangiectasia (1), DiGeorge Syndrome (1), and Charge Syndrome (1). One patient with PID (IRAK-4 deficiency) had suffered from recurrent IPD or previous hospitalizations, and only one patient with PID (DiGeorge syndrome) developed severe respiratory infections after diagnosis. In addition, a patient with partial Chromosome 16 trisomy and recurrent pneumonias had low numbers of switched-memory B cells and high numbers of CD21hi+ cells, and one patient, whose brother had been diagnosed with STAT3 negative AD Hyper IgE syndrome, had high IgE levels (996.00 UI/mL) and eosinophilia. Conclusions. PID may be the cause of 20% of pediatric patients with IPD. Prompt diagnosis and treatment after one episode of hospitalization for IPD, in the absence of previous severe and/or recurrent infections, protect against severe posterior infections in patients with PID.

P.D4.05.16
Sex and pathogen influence on monocyte activation - A special feature arising from Entamoeba histolytica infection
J. Sellos1, M. Gironegre2, S. Hoenow2, B. Krausel1, C. Marggraf1, J. Diekhoff1, H. Ittrich1, J. Jacobs1, H. Lotter1; 1Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany, 2Department and Clinic for Diagnostic and Interventional Radiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Men and male mice are more prone to develop amebic liver abscess (ALA) following infection with the parasite Entamoeba (E.) histolytica. In the murine model for the disease, a CCL2-dependent recruitment of Ly6C+ monocytes and TNFα is responsible for tissue damage. Interestingly, in E. histolytica infected human, men show higher CCL2 levels compared to women suggesting a similar mechanism in humans may be implicated in the human disease. Here, we analyzed sex-specific mechanisms in monocytes in response to E. histolytica. E. histolytica-dependent liver destruction in mice was analyzed by magnet resonance imaging (MRI), monocytes were characterized by FACS and serum cytokines by using a multiplex assay. Human macrophages or monocytes were stimulated with various antigens (LPS, LTA, E. histolytica) or conditioned medium, supernatants were analyzed using multiplex assays and cells were characterized by FACS. Male mice showed larger abscess lesions, higher blood and liver CD11b+ Ly6C+ monocyte frequencies as well as an altered cytokine profile in comparison to female mice. CCL2-dependent recruitment of Ly6C+ monocytes in ALA led to an increased population of CCR2-expressing CD14+ monocytes in men, but not in women. Inflammatory monocytes play an important role in inducing a sex difference in the outcome of ALA in mice. We were able to generate a comparable inflammatory phenotype monocyte population in vitro in model system with the same sex difference, which might be able to induce a similar sex bias.

P.D4.05.17
Dynamics and trafficking of mGBP protein complexes within membranous compartments during infection with Toxoplasma gondii
N. Steffens, L. Legewit, E. Kravets, D. Degrandi, K. Pfeffer; Medical Microbiology and Hospital Hygiene, Düsseldorf, Germany.

Introduction: During invasion of target cells, Toxoplasma gondii (T. gondii) creates a paracystophorous vacuole (PV). Members of the murine guanylate binding protein family (mGBPs) assemble at the cytosomeplasmic side of the PV and interact with this membranous compartment via yet uncharacterized mechanisms.

Objectives: This analysis aims at unravelling the molecular mechanisms by which mGBPs impair the vital functions of T. gondii.

Methods: Stable cell lines expressing fluorescent mGBPs were generated and infected with GFP-/mCherry-expressing T. gondii to analyze the dynamics of mGBPs via Confocal Live Cell Imaging. Super-resolution technologies were used to analyze the doubly transduced cell lines during infections in detail. Giant unilamellar vesicles technology (GUV) is in progress to investigate the binding capacity of mGBPs to membranes and their modulation of membrane integrity. Also, cytosolic compartments of mGBPs (vesicle-like structures, VLS) and the T. gondii-PV will be characterized by electron microscopy.

Results: First results suggest that mGBP localizes in VLS after interferon-γ stimulation. Furthermore, mGBP7 colocalizes with mGBP3 and partially with mGBP6 and mGBP8 but without mGBP2. mGBP1 appears to recruit the PV compared to mGBP7. Additionally, mGBP7, mGBP3, mGBP6 and mGBP2 are able to accumulate directly at the plasma membrane of T. gondii, subsequently leading to parasite death.

Conclusion: mGBPs belong to a family of GTPases that can interact with the T. gondii-PV and selected members directly attack the parasite membrane. The analysis of the mechanisms will help to understand Toxoplasmosis and to find new treatment opportunities in the long term.
Immunological response of people living with Human Immunodeficiency Virus on highly active antiretroviral therapy in Senegal

M. D. SYLLA NGAWA, B. Nibengue, M. Mbou, A. Sylva, S. Atsou1, R. Derwache2, T. N. Dieye3, A. Dieye1

1University Cheikh Anta Diop of Dakar - SENEGAL, Dakar, Senegal; 2General Hospital of Grand-Yoff, Dakar, Senegal.

Introduction: The objective of this study was to evaluate the immunological-virological response of HIV-infected patients by analyzing the peripheral CD4 lymphocytes rates and viral load following highly active antiretroviral therapy (HAART).

Material and methods: A cross-sectional prospective study was conducted from January to September 2017. The study population included patients living with HIV (PHLV) referred to General Hospital of Grand-Yoff (HOGOY). The analysis focused on socio-demographic, clinical, and biological parameters. Data were analyzed by descriptive and analytical statistical methods using Statview 5.0 software.

Results: The cohort of PHLV was composed of 127 patients. The median age at diagnosis was 41 years. The circumstances of diagnosis were dominated by opportunistic infections (47.3%), HIV-1 infection was predominant (88%). The most represented therapeutic combinations were Tenodan / Efavirenz (46% of patients) and Combivir / Efavirenz (27.8%) with 44 months as average length of HAART. Following HAART, the median TCD4 counts has increased from 293 cells/µl at diagnosis to 401 cells/µl. The progression of TCD4 median was significant (p < 0.0001) only for PHLV 1 under HAART. Among treated patients, 22.8% underwent an immune restoration during the follow-up whilst 30% had immunological failure.

The immunological discordance was 23.6%. For PHLV with detectable viral load, there was no significant difference between the initial viral load and the viral load after treatment.

Conclusion: These data allow to optimize existing treatments and contribute, through a multidisciplinary care, to improve the patient survival.

Immunovirological response of people living with Human Immunodeficiency Virus on highly active antiretroviral therapy in Senegal

Ageing outweighs the impact of ART regimen on the immune restoration of virally suppressed long-term treated HIV patients

R. Emilova1, V. Todorova2, N. Vacheva2, Y. Strashimir2, I.tenke2, I. Alexiev1, M. Nikolova2

1NRRI of Immunology, National Center of Infectious and Parasitic Diseases, Sofia, Sofia, Bulgaria; 2Specialized Hospital for Active Treatment of Infectious and Parasitic Diseases, Sofia, Bulgaria

Life-long combined antiretroviral therapy (cART) is the only current strategy in HIV-patients. The factors impacting residual immune activation and long-term prognosis have not been fully elucidated. Aim: To evaluate the cumulative impact of cART regimen and ageing on the immune restoration in virally suppressed long-term treated HIV+ patients.

Material & Methods: Data for 182 HIV+ patients (52 F; 130M), mean age 43 (24-76), on continuous cART for at least 5y, with SVR (HIVVL<2.0 log.,copies/ml) after the second year were analyzed. Subgroups according to regimen were: INSTI-based (n=14), NNRTI-based (n=28), LPV-based (n=89), DRV-based (n=54). Percentage and absolute counts (AC) of CD4, CD8, CD4: CD8, CD4+DTP and CD4-CD8-DTP were determined by flow cytometry, MFI and transmission category on immune restoration was evaluated by multiple regression analysis (SPSS21). Results: CD4AC and CD4/CD8 ratio did not differ between subgroups at 2y (p>0.05), and significantly at 5y of treatment (mean 629±5,19, and 0.74±0.05, p<0.0001). CD4AC and CD4:CD8 restoration were not affected by regimen (R2=0.01 and, R2=0.003, p>0.05). Age was the most important predictor for CD4/CD8 restoration (0.12vs.0.20, F=8.30, p<0.029).However, LPV-based cART was associated with lower level of DIFT reflecting low thymic activity of DIFT (F=17.154, p<0.01). In addition, advancing age had a negative effect on the long term regulation of immune activation.(109yrs. 171, F=15.02, p<0.01). Age is a major factor for immune restoration in long-term treated HIV patients. Old generation PI may directly impact the regulation and activation of T cells and should be avoided in the elderly patients.

Extracellular matrix degradation is regulated by an acidic inflammatory microenvironment

A. M. Whittington1, D. E. Kiwara1, F. S. Turner1, R. H. Gillman1, J. S. Friedland1

1Imperial College, London, United Kingdom; 2Edinburgh Genomics, University of Edinburgh, Edinburgh, United Kingdom; 3Johns Hopkins University, Baltimore, United States.

Background: The inflammatory microenvironment is acidic. Extracellular pH at sites of Mycobacterium tuberculosis (M.tb) infection nears 7.0 compared to physiological pH 7.4. Acidosis is caused by pH-sensitive G-Protein coupled receptors (TDAG6, OGR1, and GPR12). Tuberculosis is characterised by marked inflammation and tissue destruction driven by host derived Matrix Metalloproteinases (MMPs). This study investigates how extracellular acids modulates immune responses in TB.

Methods: Transcriptomic profiling of primary human monocyte-derived macrophages (MDMs) infected with virulent M.tb H37Rv at pH 7.4 or with acidosis (pH 7.0) was performed by RNA-seq. MDM expression of acidosis receptors was determined by qPCR and immunohistochemistry of biopsies from TB patients. Protein secretion by infected MDMs was measured by Luminex.

Results: Acidosis produces system level transcriptional change in M.tb infected MDMs with 2616 genes upregulated and 2919 downregulated at pH 7.0 compared with 1556 by RNA-seq. MDM expression of acidosis receptors was determined by qPCR and immunohistochemistry of biopsies from TB patients. Protein secretion by infected MDMs was measured by Luminex.

Conclusions: The acidic microenvironment enhances tissue degradation pathways and MMP secretion in TB. TDAG-8 and OGR-1 are potential novel targets for host directed therapy.
P.D4.06.03  Cryptic high mannose self-recognition by macrophage Mannose Receptor is a new strategy to identify and characterize the novel strategy to identify and characterize the immune response to Ascaris spp., the most common of the soil-transmitted helminths. The large roundworms infecting humans and pigs, A. lumbricoides and A. suum, are genetically almost identical and cross-transmission occurs, highlighting the role of pig studies to understand the host-parasite interplay. The quality of an immune reaction largely depends on a relatively small fraction of antigen-specific CD4+ T-cells that orchestrate the adaptive immune response. Our study therefore aimed to investigate frequency, phenotype and specificity of Ascaris-reactive CD4+ T-cells in the pig as a natural host. We used CD40L (CD154) expression as an early TCR activation marker of swine CD4+ T-cells to study the development of an Ascaris-specific CD4+ T-cell pool. Our data demonstrates the onset of a robust, antigen-specific T-cell response already during larval migration. Ascaris-specific CD4+ T-cells are directed against excretory-secretory-proteins and parasite lysates. To improve functional analysis of antigen-specific lymphocytes we adapted the method of antigen-reactive T-cell enrichment (ARTE) to porcine, parasite-specific CD4+ T-cells. Enrichment analysis revealed phenotypic differences in CD4+ T-cells from peripheral vs. migration affected compartments, such as lung parenchyma. Our approach thereby offers a novel strategy to identify and characterize Ascaris-specific CD4+ T-cells directly in the pig, and will be used to unravel mechanisms of protection and protective antibodies.

P.D4.06.04  Helminth-specific CD4+ T-cell responses during Ascaris infection in the pig

F. Ebner, J. Schlasser, L. Tedin, S. Hartmann; Institute of Immunology, Center for Infection Medicine, Department of Veterinary Medicine, Berlin, Germany.

The pig represents the ideal human-relevant model system to study the immune response to Ascaris spp., the most common of the soil-transmitted helminths. The large roundworms infecting humans and pigs, A. lumbricoides and A. suum, are genetically almost identical and cross-transmission occurs, highlighting the role of pig studies to understand the host-parasite interplay. The quality of an immune reaction largely depends on a relatively small fraction of antigen-specific CD4+ T-cells that orchestrate the adaptive immune response. Our study therefore aimed to investigate frequency, phenotype and specificity of Ascaris-reactive CD4+ T-cells in the pig as a natural host. We used CD40L (CD154) expression as an early TCR activation marker of swine CD4+ T-cells to study the development of an Ascaris-specific CD4+ T-cell pool. Our data demonstrates the onset of a robust, antigen-specific T-cell response already during larval migration. Ascaris-specific CD4+ T-cells are directed against excretory-secretory-proteins and parasite lysates. To improve functional analysis of antigen-specific lymphocytes we adapted the method of antigen-reactive T-cell enrichment (ARTE) to porcine, parasite-specific CD4+ T-cells. Enrichment analysis revealed phenotypic differences in CD4+ T-cells from peripheral vs. migration affected compartments, such as lung parenchyma. Our approach thereby offers a novel strategy to identify and characterize Ascaris-specific CD4+ T-cells directly in the pig, and will be used to unravel mechanisms of protection and protective antibodies.

P.D4.06.05  A comparison of selected immunological parameters in cyclophosphamide treated C57BL/6 and C3H mice immunized with BCG or rBCGm-IL-18

M. Wlodarczyk, A. Bednarek, J. Kowalska, M. Bromirska, M. Druszczynska, M. Kowalewicz-Kulbat, A. Krupa, W. Rudnicka, M. Fai, Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland.

The work addressed the question of potentially superior immunogenicity of recombinant M. bovis BCG producing mouse IL-18 compared to the parent strain BCG under immunosuppressive conditions in the context of different sensitivity of mice strains to mycobacterial infection (susceptible C57BL/6 and resistant C3H mice). Mice were intradermally immunized with BCG or rBCGm-IL-18 and after 6 weeks intraperitoneally treated with cyclophosphamide (CP, 50mg/kg b.w.) for 7 days. The following specimens were isolated: serum - to measure the concentration of selected cytokines (Bio-Plex Pro)-Assay), alveolar macrophages - to investigate the effectiveness of phagocytosis (acidine orange and acridine orange stained cytospins) - to determine the expression of CD34 and CD117. The mean percentage of alveolar macrophages involved in phagocytosis, isolated form control C57BL/6 mice, both immunocompetent and immunosuppressed was similar, and it was significantly higher than in control C3H mice. Both BCG and rBCGm-IL-18 immunization caused a decrease in the phagocytic activity of macrophages, however the increase in the killing effectiveness was observed. C3H mice were more efficient producers of IL-2, IL-10, IL-12 and GM-CSF, but not TNF-α, than C57BL/6 mice. The impact of immunosuppression or type of BCG strain was moderate at most and ambiguous. There were not significant differences regarding the level expression of CD34 as well as CD117 on bone marrow cells between the mouse strains. The use of CP and recombinant BCG strain did not significantly affect the expression of the receptors. This work was supported by the National Science Centre (Poland) under Grant number 2013/11/B/NZ6/01304.

P.D4.06.06  Complex interplay between Ly49 receptors and cytomegalovirus

V. Jurancic Lincic, J. Zeleznjak, B. Popovic, B. Lincic, M. Babic, M. Cesarec, A. Halenius, I. Daeklen, A. Krimpotic, S. Jonic; Faculty of Medicine, Rijeka, Croatia; Institute of Virology, Universitätsklinikum, Freiburg, Germany; Institute of Virology, Wurzburg, Germany.

Chimpanzees not only are the closest human relatives but also the only non-human species whose lymphocytes have Ly49 receptors. In order to identify such receptors in other species we have focussed on the pig. Ly49 receptors are mainly involved in NK cell receptors in order to trigger the NK cell-mediated "missing-self" recognition, murine cytomegalovirus (MCMV) encodes m40/gp34, a protein which binds to a portion of MHC I molecules back to the cell surface enabling them to engage binding to inhibitory Ly49 receptors (Ly49h). However, m40 binds only a small portion of MHC I to the surface. We have identified and characterized 11x0 viral protein Mat1p encoded by the MCMV's most abundant transcript (MAT) that helps in the phagocytic activity of macrophages, however the increase in the killing effectiveness was observed. CD34+ mice were more efficient producers of IL-2, IL-10, IL-12 and GM-CSF, but not TNF-α, than C57BL/6 mice. The impact of immunosuppression or type of BCG strain was moderate at most and ambiguous. There were not significant differences regarding the level expression of CD34 as well as CD117 on bone marrow cells between the mouse strains. The use of CP and recombinant BCG strain did not significantly affect the expression of the receptors. This work was supported by the National Science Centre (Poland) under Grant number 2013/11/B/NZ6/01304.

P.D4.06.07  Chitinase 3-like 1 protein (CHI3L1) plays a critical role in RSV-induced airway inflammation


Background: Chitinase 3-like 1 protein (CHI3L1) [YKL-40 in humans and breast regression protein (BRP)-39 in mice] is required for optimal allergen sensitization and Th2 cell responses during larval tissue migration. Conversely, blocking this protein leads to a robust, antigen-specific Th2 response already during larval tissue migration. Ascaris-specific CD4+ T-cells are directed against excretory-secretory-proteins and parasite lysates. To improve functional analysis of antigen-specific lymphocytes we adapted the method of antigen-reactive T-cell enrichment (ARTE) to porcine, parasite-specific CD4+ T-cells. Enrichment analysis revealed phenotypic differences in CD4+ T-cells from peripheral vs. migration affected compartments, such as lung parenchyma. Our approach thereby offers a novel strategy to identify and characterize Ascaris-specific CD4+ T-cells directly in the pig, and will be used to unravel mechanisms of protection and protective antibodies.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 489
POSTER PRESENTATIONS

P.D4.06.08 Targeting Inhibitory Receptors LAG-3 and TIM-3 to Enhance Anti-parasitic CD4+ T cell Responses in Visceral Leishmaniasis

R. Kumar1, N. Singh2, B. Singh3, C. Engwerda4, S. Sundar5;
1Institute of Science, Banaras Hindu University, Varanasi, India, 2Institute of Medical Science, Banaras Hindu University, Varanasi, India, 3QIMR Berghofer Medical Research Institute, Brisbane, Australia.

Introduction: CD4+ T cell exhaustion is a common phenomenon during chronic visceral leishmaniasis (VL) which causes reduced IFN-γ secretions, critical for controlling the parasite replication. This can be mediated by abnormal expression of immunosuppressive receptors LAG-3 and TIM-3 on the surface of CD4+ T cells. The present study aims to investigate the role of LAG-3 and TIM-3 in patients with VL.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from VL patients before and after drug treatment. CD4+ T cells were enriched using magnetic beads. Ex-vivo mRNA expression of TIM-3 and LAG-3 was measured in both PBMCs as well as enriched CD4+ T cells by Real-Time PCR and surface expression were examined by flow cytometry. To know the interplay between metabolic processes and innate immune response against leishmaniasis, significantly enriched pathways in infected DCs. On the other hand, DCs exposure to fixed parasite enhanced the expression of genes related to cytokine-cytokine receptor interaction and may play an important role in the innate and adaptive immunity against this fungal pathogen.

Conclusion: We observed enhanced mRNA expression of TIM-3 and LAG-3 in whole PBMCs as well as CD4+ T cells of VL patients in pre-treatment stage and TIM-3 in expanded to post treatment as well as enhanced surface expression as revealed by flow cytometry analysis. We observed an enhanced IFN-γ secretion in whole blood culture after LAG-3 blockade compared but there was no any effect of TIM-3 blockade on IFN-γ secretion. These results identify LAG-3 as an important immunotherapeutic target to enhance anti-parasitic CD4+ T cell response and treat VL patients.

P.D4.06.09 Protagonulin-1 In Respiratory Syncytial Viral bronchial cells

K. RUMAWAT1, L. Brouwer1, S. Feni1, A. Petersen2, B. Lambrecht2, M. Nawijn1, G. Koppelman3, L. Meyard1, L. Bont4;
1University Medical Center Utrecht, Utrecht, Netherlands, 2Oncode Institute, University Medical Center Utrecht, Utrecht, Netherlands, 3University Medical Center Groningen, Groningen, Netherlands, 4VIB Center for Inflammation Research, Ghent, Belgium, 5Vib University, Ghent, Belgium.

Early life Respiratory syncytial viral (RSV) bronchitis is linked to declines in developmental and asthma in later life. SNPs in Protagonulin-1 (PDCD1), an airway epithelial surface protein, have been associated with increased susceptibility to airway hyperresponsiveness (AHR). Since aberrant AHR indicates diminished lung function and is a key feature of RSV bronchitis, we investigated whether PDCD1 could play a role in RSV disease.

Human bronchial epithelial cell line, 16HBE, was infected with RSV-A2 and PDCD1 expression was measured by qPCR and western blotting. C57BL/6 wild-type (WT) and PDCD1 knockout (KO) mice were infected with RSV-A2. Body weight loss was monitored as clinical parameter for RSV disease. On 5-day post-infection (dpi), lung inflammation was determined by broncho-alveolar (BAL) fluid analyses and lung function was measured by Flexivent. In vitro, RSV-A2 infection decreased PDCD1 protein expression in 16HBE cells with variable effects on mRNA. In vivo, RSV-A2 infection led to body weight loss and showed increased viral titres in the BAL fluid, indicating an ongoing RSV disease. Moreover, BAL fluid showed significantly increased cellular influx to the lungs. Furthermore, RSV infection augmented AHR in response to methacholine. There were no differences between WT and KO mice in weight loss, lung inflammation, AHR or viral replication post-RSV infection. In conclusion, we confirm RSV causes airway inflammation and hyperresponsiveness in adult mice, but these effects are independent of PDCD1. Ongoing studies aim to define the role of PDCD1 in the occurrence of RSV-enhanced allergic airway inflammation.

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P.D4.06.10 Adaptation to complex host niches drives resistance to neutrophils by fungal pathogen C. albicans

J. Lopes1, E. Backman2, S. Holmberg3, M. Stylianou4, J. Jass5, R. Caesson6, C. F. Urban7;
1Department of Clinical Microbiology, Umeå University, Umeå, Sweden, 2The Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå, Sweden, 3School of Science and Technology Örebro University, Örebro, Sweden, 4Section Molecular Periodontology, Department of Odontology, Umeå University, Umeå, Sweden.

Immun systems have developed to prevent harm inflicted by other organisms. As successful colonizers, these effector mechanisms have evolved traits to escape from immune attack. Candida albicans is a yeast that is isolated from humans and its survival is connected to the ability to adapt to stress or nutrient scarcity within the host. This environment shapes the niche for C. albicans. The yeast has evolved to escape environmental clues by predicting and adapting to secondary stimuli, thereby improving the organism’s fitness. In this case the niche shapes the environment. Here, we describe one such event. Upon infection, neutrophils are rapidly infiltrating into the mucosal niche. Circular neutrophils are effective phagocytes which serve as first line defense against fungal pathogens. High numbers of infiltrating cells coupled with the formation of microbial structures, such as biofilms, lead to induction of host monocytes/macrophages. This increases the character of effect of anaemia on neutrophil responses encountering C. albicans both under the form of different planktonic morphotypes and under biofilm growth. We found that a persistent anaemia milieu did not affect neutrophil function, however, hampered neutrophil responses towards C. albicans. PAMP sensing and subsequent responses against C. albicans were reduced under anaemic conditions allowing the yeast to escape from neutrophil attack. In addition, anaemia contributed to increased fungal growth, a trait we found to be conserved in many Candida species. We therefore conclude that adaptation to low oxygen is not only an evolutionary advantage but rather a pre-requisite for successful colonization and infection of the host.

P.D4.06.11 The Syk-Coupled C-type lectin receptors Dectin-2 and Dectin-3 are involved in Paracoccidioides brasiliensis recognition by human plasmacytoid dendritic cells

1Department of Immunology, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil, 2Instituto de Ciência e Tecnologia, Universidade Federal de São Paulo, São José dos Campos, Brazil.

Plasmacytoid dendritic cells (pDCs), which have been extensively studied in the context of the immune response to viruses, have recently been implicated in host defense against fungal pathogens. In this context, we decided to characterize the innate immune receptors involved in the interaction between pDCs and yeast cells. Purified pDCs were obtained from peripheral blood mononuclear cells from healthy donors and they were stimulated with P. brasiliensis with or without blocking antibodies to innate receptors. We demonstrated that P. brasiliensis stimulation activates pDCs that inhibit fungal growth and secrete pro-inflammatory cytokines and type I IFNs. Importantly, we also demonstrated that dectin-2 and dectin-3 are expressed in pDCs and appear to be involved (via Syk signaling) in the pDC-Yeast interaction. Thus, the present study aims to investigate the role of pDCs and yeast-specific receptors in P. brasiliensis recognition and may play an important role in the innate and adaptive immunity against this fungal pathogen.

P.D4.06.12 Transcriptional profiling of Leishmania infantum infected dendritic cells: insights into the role of immunometabolism in host-pathogen interactions

M. Margaron1, M. Agallou1, D. Karagouni2, A. Hatzigeorgiou1, E. Karagouni1;
1Laboratory of Cellular Immunology, Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece, 2Diana-Lab, Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece, 3Diana-Lab, University of Thessaly, Volos, Greece.

Leishmania parasites are the causative agents of leishmaniasis, a group of diseases that range in manifestations from skin lesions to fatal visceral disease. Dendritic cells (DCs) hold the key role in orchestrating immune responses against leishmaniasis by regulating the activation of adaptive immunity. The aim of the present study was to investigate changes in the whole transcriptome of murine bone marrow-derived DCs infected with Leishmania infantum using murine bone marrow-derived RNA-seq. DCs exposure to parasite resulted in infection of almost 50% of DCs. The infected DCs were sorted using flow cytometry and whole RNA was isolated for transcriptome analysis. According to RNA-seq results, we identified 712 differentially expressed genes (DEGs) in DCs infected with L. infantum (352 up-regulated and 366 down-regulated). Comparative analysis of DEGs in infected DCs compared to DCs exposed to chemically inactivated parasites, revealed that twice as many DEGs were more abundant during infection demonstrating the influence of parasite infection on host gene transcription. KEGG pathway analysis revealed that metabolic pathways, including glycolysis, electron transport chain and fatty acid degradation were among the most significantly enriched pathways in infected DCs. On the other hand, DCs exposure to fixed parasite enhanced the expression of genes related to cytokine-cytokine receptor interaction and antigen processing. These data provided insights into the molecular mechanisms underlying L. infantum infection of DCs and might extend the knowledge regarding the interplay between metabolic processes and innate immune response against leishmaniasis.

This work was funded by the NSRF 2014-2020 and co-financed by Greece and the European Union (MIS 5002486).
**Poster Presentations**

**P.D4.06.13**
Target specific design and synthesis of a novel water soluble ferrocenyloquinoline derivative as potential leishmanial agent

D. Mukherjee1, S. Dey1, M. Yousaf1, S. Chakraborty2, A. Choudhuri1, A. Dutta1, A. Hussain1, S. Chakraborty1, S. Adhikari1, C. Pafi2.
1Department of Zoology, West Bengal State University, Barasat, Kolkata, India. 2PG Department of Zoology, Barasat Government College (Present Address), Barasat, Kolkata, India.

Background: Visceral Leishmaniasis (VL), a neglected parasitic disease caused by Leishmania donovani, is responsible for severe health problems in India. Despite of significant progress in anti-leishmanial research, ultimate introduction of novel, safe and cost-effective drugs is far away from agreeable destination. This failure is majorly attributed to poor water solubility of drugs, in consequence, oral administration becomes challenging. Thus, the development of water-soluble oral drugs with low manufacturing prices remains highly desirable to treat VL.

Methods: We adopted quaternization strategy, where 7-Chloro-N-[2-(1H-5-ferroceny-1,2,3-triazol-1-yl)-ethyl] quinolin-4-amine was modified to yield 7-Chloro-quinolin-4-yl-methyl-[2-(4-ferroceny-[1,2,3]triazol-1-yl)-ethyl] aminochloride (CQFCWS) and tested the anti-leishmanial efficacy, both in vitro and in vivo (oral and intramuscular administration in BALB/c mice).

Results: CQFCWS was highly efficient at very low IC50 concentrations and was nontoxic towards host splenocytes, in vitro and in vivo. CQFCWS maintained hematopoietic bone marrow cell proliferation, in situ and did not alter Phase I and Phase II detoxification enzyme components in host liver. Immunomodulating potential of CQFCWS was confirmed by its ability to skew Th2 response towards Th1. CQFCWS did not induce drug resistance genes (MDR1 and MRP1) in L. donovani, in vitro. CQFCWS acted through a putative target and limited the expressions of L. donovani survival enzymes, trypanothione reductase and ornithine decarboxylase. To explore the binding efficiency of CQFCWS to trypanothione reductase, molecular docking followed by dynamic simulation was performed. CQFCWS could also induce apoptosis by upregulating PARP1 and downregulating SIR2 in parasites.

Pharmacokinetic study regarding the bioavailability of CQFCWS revealed a short elimination half-life of the drug.

**P.D4.06.14**
The role of TAM receptors, and their ligand, Gas6, in resistance and susceptibility during ZIKV infection

L. G. Oliveira, N. G. Zangulga, C. M. Polonia, C. L. Freitas, J. S. Peron; Universidade de São Paulo, São Paulo, Brazil.

Introduction. Zika virus (ZIKV) has gained worldwide attention as it has been correlated with severe fetal malformations, causing the Zika Congenital Syndrome (ZCS). However only 6-12% of mothers infected with ZIKV give birth to babies with malformations. These observations suggest that ZIKV infection during pregnancy is not deterministic for ZCS, but other susceptibility factors might be involved. The viral entry receptors are important candidates. Tyro3, Axl, Mertk (TAM receptors), and their ligands, Gas6 and Protein S are important for ZIKV internalization and can facilitate viral entry by bridging viral envelope phosphatidylserine in a mechanism called viral apoptotic mimicry. Although, their correlation with resistance or susceptibility to infection is largely unknown. Objective. Evaluate the immunobiology of TAM receptors, and their ligand, Gas6, in resistance and susceptibility to ZIKV infection. Results. We observed that SJL, susceptible mouse lineage, showed higher levels in mRNA expression of Tyro3, Axl and Gas6 compared with C57BL/6, resistant lineage. In this context, we demonstrated that the combination of rmGas6+ZIKV in SJL and C57BL/6 infection increase the viral load in spleen while the use of Axl kinase blocker, R428, decrease the amount of viral particles. Interestingly, the use of rmGas6+ZIKV led to the development of C57BL/6 affected offsprings, turning this lineage susceptible to ZCS. Conclusion. Our results suggest the crucial role of TAM receptors, and the intracellular kinase portion of Axl, during ZIKV infection. These data contribute for a better knowledge about the invasion mechanisms of ZIKV, in vivo, that could be involved in the ZCS.

**P.D4.06.15**
Virulent Salmonella enterica serovar Typhimurium modulates the production of neutrophils extracellular traps

B. M. Schultz1, S. P. Muraro1, G. Fabiana de Souza2, S. Dias de Oliveira1, B. N. Portor3, S. M. Buenor2.
1Pontificia Universidad Católica de Chile, Santiago, Chile. 2Millennium Institute on Immunology and Immunotherapy, Santiago, Chile. 3Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil.

Salmonella enterica serovar Typhimurium is an important cause of gastrointestinal diseases worldwide. To infect the host, S. Typhimurium has several virulence factors encoded in chromosomal cluster known as Salmonella Pathogenicity Islands (SPI). It has been described that S. Typhimurium infected cells secrete anti-inflammatory cytokines, promoting a tolerogenic environment. During this infection, the innate immune response triggered by the bacteria is the migration of neutrophils to the site of infection. These cells clear the infecting microorganisms through different mechanisms, such as neutrophils extracellular traps (NETs). However, innate immune response is not enough to avoid S. Typhimurium dissemination. For this reason, it is important to evaluate the immune response mediated by neutrophils against S. Typhimurium. We isolate human blood or mice bone marrow derived neutrophils and induce the production of the NETs at different multiplicity of infection. 1x10⁶ cells were infected for 10 or 180 minutes, in order to evaluate if the S. Typhimurium induce NETs in the early or classical way, respectively. We also performed inhibition assays, to evaluate the NETs pathways production. We found that human blood and mice bone marrow derived neutrophils infected with a S. Typhimurium induce NETs production in a MOI dependent manner and by the classical way. Interestingly, a minimal MOI 1:1 induces NETs production, however, the bacteria has virulence factors that could be related with the suppression of the immune response, which favor the bacterial dissemination.

**P.D4.06.16**
The effect of hookworm co-infection on pneumococcal carriage and invasive disease


Background and aims: hookworm infections are highly prevalent in sub-Saharan Africa and South-East Asia, where the incidence of invasive pneumococcal disease is also high. Hookworms are gastrointestinal nematodes that burrow their host for months or years, causing chronic infection. In addition, hookworm larvae migrate through the lung, which is associated with significant tissue damage. Chronic helminth infections promote an immunoregulatory environment, which is associated with increased numbers of T regulatory (Treg) cells and higher levels of the immunosuppressive cytokines, TGF-β and IL-10, which can alter the immune response to bystander pathogens. Resistance to pneumococcal disease is based upon a delicate balance between Treg-driven immune tolerance and pro-inflammatory responses which may clear infection but can also lead to tissue damage, providing a route for bacterial dissemination. Thus, the tissue damage caused by larval migration through the lung and the immunoregulation associated with adult hookworms in the small intestine may influence pneumococcal disease progression.

Methods: We are currently investigating the effect of hookworm (Nippostrongylus brasiliensis and Heligmosomoides polygyrus) co-infection on pneumococcal disease progression using mouse models of pneumococcal carriage and pneumonia.

Results: Our data suggest that both lung migration and chronic gastrointestinal infection caused by these helminths lead to increased mortality in mouse pneumococcal pneumonia models and may also promote bacterial seeding from the nasopharynx to the lungs in pneumococcal carriage models. Conclusions: These studies suggest that hookworm co-infections can worsen disease outcome in pneumococcal pneumonia and promote progression from asymptomatic carriage to invasive disease.

**P.D4.06.17**
Leishmania donovani: CD2 biased immune response skew s the SAG mediated therapy for a predominant Th1 response in experimental infection

S. Sinha, S. Sondaram; Centre for Biotechnology, University of Allahabad, Allahabad, India.

We have evaluated the effect of combining CD2 with conventional antimonial (sb) therapy in protection in BALB/c mice infected with either drug sensitive or resistant strain of Leishmania donovani with 3x10³ parasites via-intra-cardiac route. Mice were treated with anti CD2 adjunct SAG sub-cutaneously twice a week for 4 weeks. Assessment for measurement of weight, spleen size, anti-Leishmania antibody titer, T cell and anti-leishmanial macrophage function was carried out day 0, 10, 22 and 34 post treatments. The combination therapy was shown boosting significant proportion of T cells to express CD25 compared to SAG monotherapy. Although, the level of IFN-gamma was not statistically different between combination vs monotherapy (p = 0.396) but CD2 treatment even alone significantly increased IFN-gamma production than either SAG treatment (p = 0.045) or with CD2 adjunct SAG treatment (p = 0.005) in Ld-5 strain as well as in Ld-R strain. The influence of CD2 adjunct treatment was also documented in anti-leishmanial functions in macrophages.Unlike SAG treatment, SAG with CD2 also led to production of nitric oxide and TNF-a, resulting in resulting in most effective clearance of L. donovani from infected macrophages. Our results indicate that CD2, which can boost up a protected Th1 like response, might also be beneficial to enable SAG to induce Macrophages to produce more INF-γ and hence control the infection in clinical situation like Visceral Leishmaniasis.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

491
**POSTER PRESENTATIONS**

**P.D4.06.19**

**Protective effects of soluble human CDS in experimental fungal sepsis**

M. Velasco-de Andrés1, M. Martínez-Flores2, C. Catalá3, J. Simón4, E. Carreras1, O. Zaragoza1, F. Lazano1,2,3

1Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, 2Mycolgy Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III (ISCIII), Majadahonda, Spain, 3Servei d’Immunologia, Centre de Diagnòstic Biomèdia, Hospital Clinic de Barcelona, Barcelona, Spain, 4Department of Biomedecine, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain.

**Sepsis due to invasive fungal infections (IFIs) are an emerging problem worldwide related to the widespread adoption of aggressive immunosuppressive therapy among certain patient groups, and the increasing use of invasive surgical interventions. Fungal recognition receptors on a series of soluble or membrane-bound receptors (e.g. C-type lectins, scavengers or TLRs) expressed by host innate and/or adaptive immune cells, which could have therapeutic usefulness. Previous work by our group showed that the ectodomain of CSF-1, a scavenger-like lymphocyte-specific surface receptor, binds to and aggregates pathogenic and saprophytic fungal cells through recognition of β-glucans, a conserved constituent of fungal cell walls. Moreover, i.p. infusion of recombinant soluble human CDS (rhCDS) protein showed protective effects in a mouse model of septic shock-like syndrome induced by pneumococci, a glucan-rich particle from yeast. The present work confirms the in vivo efficacy of rhCDS infusion in two experimental models of fungal infection caused by pathogenic fungal species, namely C. albicans and C. neoformans. Following therapeutic i.v. administration of rhCDS to fungal-infected CD1 mice, significant time- and dose-dependent effects on mouse survival, body weight loss and fungal load were observed. Increased leucocyte infiltration (at expenses of relevant immune cell subsets such as NKs, cDCs, granulocytes and B cells) were also evidenced in targeted organs (namely kidney). Altogether, these results suggest the potential therapeutic value of rhCDS administration in invasive fungal infections.**

**P.D4.07 Exploiting host pathogen interaction - Part 7**

**P.D4.07.01**

The impact of T cell-derived Neuropilin-1 on immune cell infiltration and pathogen clearance during Plasmodium infection

H. Abberger, J. Buer, W. Hansen;
Institute of Medical Microbiology, University Hospital Essen, Germany.

**Malaria is induced by the parasite Plasmodium spp. which, in 2016, affected 216 million people and led to 445,000 deaths worldwide. Characteristic symptoms include fever, headache and nausea, malaise and in severe cases, obstruction of brain vessels and disruption of the blood-brain barrier resulting in peripheral immune cell infiltration into the brain. As we reported earlier, Neuropilin-1, a receptor of class III semaphorins and VEGF, mediates regulatory T cell migration into tumor tissue resulting in suppression of effector T cells accompanied by reduced anti-tumor response. Here, we aim to analyse whether T cell-expressed Neuropilin-1 also has an impact on immune cell infiltration into the brain with possible effects on destructive CD8+ T cells during cerebral malaria. For this purpose, we infected T cell-specific Neuropilin-1 deficient mice with murine Plasmodium berghei parasites and studied pathogen clearance and development of cerebral malaria. In addition, we investigated immunological mechanisms within peripheral lymphoid organs, blood and brain by flow cytometry. Results from our study give further insights into immunological processes during cerebral malaria being of particular interest for the identification of new potential therapeutic targets.**

**P.D4.07.02**

Characterization of newly established anti-human Dectin-1 monoclonal antibody

University of Tokyo School of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan.

**Dectin-1 is a C-type lectin receptor that recognizes fungal cell wall beta-glucan, and is responsible for host defense against fungal infection by producing proinflammatory cytokines. Recent reports suggest that the Dectin-1 contributes to the development of diseases such as DSS-induced colitis and house dust mite-induced allergy. Controlling dectin-1 function may regulate the inflammatory response. To obtain antigenic monoclonal antibody against human Dectin-1, we tried to prepare hybridomas by immunizing Dectin-1 KO mice with human Dectin-1 soluble protein as an antigen.**

**The newly established clone 2D9 produced mouse IgG1 kappa chain. The affinity of 2D9 to solid-phase human Dectin-1 molecule was monitored by BLItz. The association and dissociation constant were, 2.7x10^-9 M^-1s^-1 and 9.2 x 10^-1 M^-1s^-1, respectively. The KD of 2D9 was 3.4 nM. The 2D9 significantly inhibited the binding of soluble Dectin-1 to 1,3-β-D-glucan from Candida albicans. To compare the specificity of 2D9, various commercially available Dectin-1 monoclonal antibodies are applied. The binding of PE-labeled 2D9 to human Dectin-1-expressing lymphoma-transfected was not blocked with 15E2 (Biocorpor, 259931) and GE2 (GeneTex), but only by pretreatment with 2D9. While other commercial antibodies showed partially competed between 15E2 and 259931.**

**Results suggest that 2D9 is a unique antigenic monoclonal antibody specific to human Dectin-1.**

**P.D4.07.03**

In HIV primary infection, early CAR-T reduced T cell activation but failed to restore their polyfunctionality

R. Casetti1, A. Sacchi1, V. Bordoni2, E. Cinini2, C. Pinnetti2, R. Libertone1, A. Ammassari1, A. Antonini1, C. Agrati1

1National Institute for Infectious Diseases “Lazzaro Spallanzani”, Roma, Italy, 2National Institute for Infectious Diseases, Roma, Italy.

**HIV infection alters phenotype, distribution and function of y6 T cells during HIV infection. There are no body of evidence about the impact of early CAR-T on y6 T cell dynamics. HIV+ patients were divided into Early Primary Infection (EPI, Febrile: II/IV) and Late Primary Infection (LPI, Febrile: V/VI). Phenotype functional analysis of y6 T cells were performed by flow cytometry before (T0) and 6 months (T6) post therapy, y6 polyfunctional profile was assessed after specific antigens stimulation. Before therapy, higher frequency of V61 and V62 T cells was observed in LPI compared to HCD. CAR-T restored a normal V62 T cell frequency but failed to normalize V61 T cells in LPI. At T6, activation of V61 and V62 T cells was observed in both groups. Activated V61 T cells positively correlated with viral load and negatively correlated with CD4 T cell count. CAR-T significantly reduce the y6 T cell activation to level of HCD. In both groups, CD107a expressing V61 T cells was significantly lower than HCD but was restored after CAR-T. Polyfunctional profile of V61 T cells of both groups was comparable to HCD, except for CD107a+ V61 T cell subset and therapy failed to restore that subset. At T6 a lower CD107a+ IfN or TNF producing V62 T cells in EPI and a lower TNFα producing V62 T cells in LPI was observed compared to HCD. Our data show that HIV strongly impact y6 T cell immunity soon after infection and these alterations were only partially restored by therapy.**

**P.D4.07.04**

Mass cytometry analysis reveals the landscape and dynamics of CD32a+ CD4+ T cells from early HIV infection to effective CAR

S. Coudière1, N. Tchitchek1, L. Aloufi1, B. Vasin1, C. Bourgeois1, C. Gouyvard1, V. Ackett-Fenner1, C. Lecouroux1, P. Bruni1, R. Le Grand1, A. Beignier1, D. Lambotte2, B. Favier1, 1CEA-Université Paris Sud 11-INSERM U1184, 2Institute of Viral Infections and Autoimmune Diseases (IMVA), 3IDMIT Department, IBF, DWS, Fontayen-aux-Roses, France, 4Assistance Publique-Hôpitaux de Paris, Service de Medicine Interne et Immunologie Clinique, 5Groupe Hospitalier Universitaire Paris Sud, Hôpital Bichat, 6Lemerin-Bichat, 7Paris Descartes University, EA 7327, Sorbonne Paris Cité, APHP, Necker Hospital, 8Virology Department, Paris, France, 9Institut Pasteur, Department of Immunology, Unit of Antibodies in Therapy and Pathology, Paris, France, 10INSERM, U1222, Paris, France.

**CD32a has been proposed as a specific marker of latently HIV-infected CD4+ T cells. However, CD32a was recently found to be expressed on CD4+ T cells of healthy donors, leading to controversy on the relevance of this marker in HIV persistence. Here, we used mass cytometry to characterize the landscape and variation in the abundance of CD32a+ CD4+ T cells during HIV infection. To this end, we analyzed CD32a+ CD4+ T cells in primary HIV infection before and after effective combination antiretroviral therapy (cART) and in healthy donors. We found that CD32a+ CD4+ T cells include heterogeneous subsets that are differentially affected by HIV infection. Our analysis revealed that Naive (N+) central memory (CM+) and effector/memory (Eff/Mem) CD32a+ CD4+ T cell clusters that co-express CD95 and CD64 activating receptors were more abundant in primary HIV infection and cART stages. Conversely, CD64+CD4+ T cell clusters of either the TCM+ or TCM- phenotype were more conserved in the different stages HIV infection. Further, our data show that multiple abundance modifications of CD32a+ CD4+ T cell subsets occur in the early phase of HIV infection, and some of which are conserved after effective cART. Our study brings a better comprehension of the relationship between CD32a expression and CD4+ T cells during HIV infection.**

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492 Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
POSTER PRESENTATIONS

P.D4.07.05 Matrix metalloproteinase 10 plays a role in dampening the host inflammatory response to Plasmodium infection

K. Ehrtb, R. Rappoloff, N. Steck, C. M. Overa, B. R. Finlay, G. A. Grossi

1. Institute of Medical Microbiology and Hospital Epidemiology and German Center for Infection Research (DZIF), Partner Site Hannover, Hannover Medical School, Hannover, Germany. 2. Department of Oral Biological and Medical Sciences, Centre for Blood Research, University of British Columbia, Vancouver, Canada, 3. Institute for Experimental Medicine, Christian-Albrechts University of Kiel and Research Center Borstel, Borstel and Kiel, Germany. 3. Michael Smith Laboratories, University of British Columbia, Vancouver, Canada.

Plasmodium causes a variety of diseases ranging from self-limiting enterocolitis to severe systemic infections. Dependent on the sevora, 2-5% of immunocompetent individuals become infected. However, the molecular mechanisms representing a reaction Reactions to infection are incompletely understood. We identified matrix metalloproteinase 10 (MMP-10) among the highest upregulated proteases in the intestine during chronic Plasmodium Tephyrium infection of 129sv/l mice using a protease gene chip array. Upon in vitro infection a high upregulation of Mmp10 mRNA was detected in primary bone marrow-derived macrophages. Infection of primary bone marrow-derived macrophages from Mmp-10 deficient mice induced an increased proinflammatory response as observed by higher levels of MCP-1, TNF-α, INF-β, INF-γ, and nitrosative stress in comparison to infected wild type macrophages. While there was no difference in bacterial survival inside the cells at early points post-infection (6 hours and 1 day), we detected that Salmonella can survive better when MMP-10 is absent in long-term infected cells (3 days). Furthermore, filamentous growth of Salmonella was strongly increased in Mmp-10 deficient macrophages 3 days post infection. Filamentous growth can be caused by intracellular stress like nitrosative stress and might represent a survival strategy. Similarly to macrophages, Plasmodium infection of primary intestinal fibroblasts from Mmp10 deficient mice showed higher levels of MCP-1, nitrosative stress, filamentous growth, and increased intracellular survival (3 days) in comparison to infected wild type fibroblasts. In conclusion, our results show that MMP-10 plays a role in restricting Salmonella survival and dampening the host inflammatory response to infection.

P.D4.07.06 Regulation of gene and protein expression of critical factors in the etiology of plasmodium infection by the Microsporidia infection

C. Hurtado Marcos, Y. Sáez, F. Izquierdo, S. Fenoy, C. Del Aguila

Universidad San Pablo-CeU, Madrid, Spain.

Colon cancer is one of the most prevalent cancers in most countries and presents an important public health problem worldwide, with an incidence over one million new cases annually. In addition, the International Agency for Research on Cancer has identified that certain infectious agents are capable of inducing cancer in humans (18% of the global cancer burden). Based on this background, our research is trying to demonstrate the correlation between colorectal cancer and infection by Microsporidia, that are obligate intracellular parasites that cause opportunistic infections in immunocompromised patients. In previous research, it was determined that Microsporidia modulates certain immune responses by regulating the apoptosis pathway and the cell cycle, inhibiting the activation of apoptotic proteins such as caspase-3 and p53 that is a key protein in the process of malignancy of epithelial cells in the intestine, hence the interest to analyze the possible association of colon cancer and Microsporidia. So, we have studied the possible regulation of proteins involved in the regulation of apoptosis and cell cycle, critical factors related to the development of colon cancer (APC, PTEN, TGF-Beta) by Flow Citometry and the expression of certain oncogenes (RAS, KRAS, PI3K) by retrotranscription-PCR, in the microsporidia infection “in vitro” cellular models. The results obtained showed a clear correlation between the increase of the expression of the oncogenes with microsporidia infection, as well as an induction of the proteins responsible for cell cycle. It will enrich the knowledge of the colon cancer etiology and the role of microsporidia infection in this pathology.

P.D4.07.07 Role of nutritional status and energetic/lipid metabolism on outcome of tuberculosis in mice

C. La Rocca, V. Gigantioli, T. Micillo, D. Faicchia, S. Bruzandini, C. Fuso, C. Palma, G. Matarrese

1. CNR-IEOS, Naples, Italy. 2. Istituto Nazionale Tumor “Fondazione Pascale”, Naples, Italy. 3. University of Naples “Federico II”, Naples, Italy. 4. Istituto Superiore di Sanità, Rome, Italy.

Tuberculosis (TB), a chronic infectious disease caused by Mycobacterium tuberculosis (Mtbc), still causes high mortality in the world. Mtbc is an intracellular pathogen mainly harbored by macrophages, which can attack innate and adaptive host immune response for its survival. Considering that, nutritional status and energetic metabolism highly influence the host immune function, here, we investigated the role played by a reduced caloric intake and the adipocyte hormone leptin, a factor linking energy expenditure, nutritional status and immune function, in the outcome of Mtbc infection. We found that caloric restriction reduced and spleen bacterial load and immune-mediated lung damage in infected DBA2 mice. Granuloma lesions, the number of foam cells, -a sign of Mtbc-driven dysregulation of host lipid metabolism- the levels of leptin and pro-inflammatory cytokines/chemokines such as INF-γ, interleukin (il)-1, il-6 and CCL-4, were reduced in the lungs and granuloma of infected mice at CR. All this was also associated with a significant reduction in the lungs of Mtb (mammalian target of rapamycin) pathway expression, suggesting profound alterations in cell metabolism and function. Moreover, spleen cells of CR mice better restricted bacterial growth when infected in vitro with Mtbc. This capability correlated with a reduced development of foam cells and the switch to anaerobic glycolysis (Warburg effect). Our study suggests that the outcome of TB is influenced by nutritional status and host energetic/lipid metabolism; all these factors can be novel targets for host-directed therapy to TB.

P.D4.07.08 The C-type lectin receptor CLEC12A recognizes plasmodial hemozoin and contributes to cerebral malaria development

M. K. Raulf, A. Koppelhoff, N. Steck, C. M. Overall, B. R. Finlay, G. A. Grossi

1. Institute of Medical Microbiology and Hospital Epidemiology and German Center for Infection Research (DZIF), Partner Site Hannover, Hannover Medical School, Hannover, Germany, 2. Department of Oral Biological and Medical Sciences, Centre for Blood Research, University of British Columbia, Vancouver, Canada, 3. Institute for Experimental Medicine, Christian-Albrechts University of Kiel and Research Center Borstel, Borstel and Kiel, Germany, 4. Michael Smith Laboratories, University of British Columbia, Vancouver, Canada.

Malaria represents a major cause of death from infectious disease. Hemozoin constitutes a Plasmodium-derived product that contributes to disease progression of cerebral malaria. There is a gap of knowledge of how hemozoin is recognized by innate immunity. Myeloid C type lectin receptors (CLRs) encompass a large family of carbohydrate-binding receptors that act as pattern recognition receptors in innate immunity. In the present study, we investigated whether and how CLRs contribute to Plasmodium recognition and antimalarial host defense. Using a CLR-Fc fusion protein library and CLR reporter cell lines, we identified the CLEC12A (MICL) as a novel receptor suggesting plasmodial hemozoin. Dendritic cell/T cell co-culture assays indicated that the CLEC12A/hemozoin interaction enhanced CD8+ T cell cross-pinning. Using the Plasmodium berghei ANKA mouse model of experimental cerebral malaria (ECM), we found that CLEC12A deficiency protected mice from ECM, as evaluated by a reduced survival, ameliorated clinical symptoms, and modulated T cell effector functions. In conclusion, we have identified CLEC12A as an innate sensor for plasmodial hemozoin. This is the first study that shows a direct recognition of a Plasmodium-derived ligand by a myeloid CLR.

P.D4.07.09 “Classical” and “non-classical” Th1 lymphocytes in tuberculosis protection


Infection with Mycobacterium tuberculosis (Mtbc) results in different outcomes ranging from pathogen clearance to severe tuberculosis (TB). Knowing immunological correlates associated with the protection of TB-exposed individuals against TB and TB patients against severe disease is important, but these correlates remain largely unknown. We have examined the features of CD4+ T cells associated with TB protection. We evaluated Th1, Th17, Th17Th1, non-classical Th1 (Th1) and polyfunctional CD4+ populations in TB patients (TBp) and Mtbc-exposed healthy individuals. The populations were identified based on intracellular cytokines (IFN-γ, IL-17, TNF-α, IL-2) and surface expression of chemokine receptors; the frequencies, numbers, differentiation and “exhaustion” status of each population were determined. The frequencies of Th17 and Th17Th1 lymphocytes were rare in both, TBp and Mtbc-exposed healthy individuals. Compared to TBp, Mtbc-exposed healthy individuals had more “non-classical” CCR6+CXCR3+CD4+ T cells; more polyfunctional TNF-α+IFN-γ+IL-2+ cells; less classical CCR6-CD4+ T cells; and more polyfunctional TNF-α+IFN-γ+IL-2+ and TNF-α+IFN-γ+IL-2- populations. In both TBp and Mtbc-exposed healthy individuals, Th1* and Th1 populations were functionally similar, but differed by their differentiation degree: Th1* population contained more low-differentiated CD27- effectors and lacked terminally differentiated CD27+CD28 effectors. TNF-α+IFN-γ+IL-2+ lymphocytes were less differentiated than TNF-α+IFN-γ+IL-2- and TNF-α+IFN-γ-IL-2 populations, however the latter were largely un-exhausted (PD-1⁻). In conclusion, the degree of Th1 helper cell differentiation rather than their quantities or functional properties represents a potential correlate of TB protection. The results suggest that effective vaccine should avoid T cell over-differentiation and are relevant to the development of new vaccination strategies for TB control. Supported by Grant RSF-15-15-00136.
Poster presentations

P.D.4.07.10

Mitochondrial fission is associated with mROS dependent microbical responses to Streptococcus pneumoniae in macrophages

M. MOHASIN1,2, A. J. Müller1, A. Ravi1,2
1Graduate Program, Faculty of Medicine, Yogyakarta, Indonesia, 2International PhD Program in Medicine, Taipei Medical University, Taipei, Taiwan

Abstract: Pneumonia is a leading cause of infection-related death and Streptococcus pneumoniae, the commonest cause, accounts for approximately one million deaths in children each year. Macrophages are key effectors of innate immune responses but the precise microbial mechanisms used to control pneumococci are incompletely characterised. Recently, we demonstrated that macrophages use mitochondrial reactive oxygen species (mROS) as a component of the microbial response and mROS are induced by microbial programmes that contribute to intracellular bacteria killing. West AP et al. reported that TLR agonists augment intracellular bacterial killing through inducing mROS. We hypothesized that mitochondrial homeostasis would be altered in response to intracellular bacteria as an important element of the microbial response to pneumococci.Methodology: Macrophages metabolic profiles, mROS generation, bacterial killing and fission/mitophagy were evaluated by XF24 analyser, flow-cytometry, gentamicin protection assay and confocal or electron microscopy/immunoblotting, respectively.Findings: S. pneumoniae significantly increased mitochondrial fission resulting in reduced mitochondrial network complexity. 12 hours after bacterial challenge, before apoptosis induction, mitochondrial homeostasis were co-localised or adjacent to an E3 ligase Parkin, phagolysosomes and intracellular bacteria but LC3B was not recruited. mROS co-localized with mitochondria that had undergone fission. Fission was reversed by PI-3K inhibitor 3-methyladenine but was not altered by the Drp1 inhibitor Mdivi-1. Pneumococci also reprogrammed macrophages metabolism from oxidative phosphorylation to glycolysis. Mitochondrial fission was associated with increased mROS production and intracellular bacterial killing. Conclusions: Modulation of mitochondrial fission is a potential cellular target with which to recapture host innate immune responses against internalized pathogens.

P.D.4.07.11

A new virus biosensor identifies CD11c+ monocytes as a cellular reservoir for Leishmania major proliferation and cell-to-cell spread at the site of infection

S. Heyde1, L. Philipson1, P. Formagioli1, E. A. Seij2, P. Bossaux1, B. Schraven1, A. J. Müller1
1Institute of Molecular and Clinical Immunology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany, 2Dynamics of Immune Responses, Institut Pasteur, Paris, France, 3Helmholtz Centre for Infection Research, Braunschweig, Germany

Abstract: the virulence of intracellular pathogens such as Leishmania major (L. major) relies on their ability to undergo cycles of replication within phagocytes, release, and uptake into new host cells. While all these steps are critical for successful establishment of infection, neither the cellular niche of efficient proliferation, nor the spread to new host cells have been characterized in vivo. We used a biosensor for measuring pathogen proliferation by intravital 2-photon microscopy and multiparameter histocytometry in the ongoing infection. We found that monocyte-derived CD11c+ cells constituted the main cell type harboring rapidly proliferating L. major. Synchronization of monocyte recruitment by adoptive cell transfer showed that high proliferating parasites preferentially underwent cell-to-cell spread, however newly recruited host cells were infected irrespectively of their cell type or maturation state. We propose that among these newly infected cells, only CD11c+ cells, most probably monocyte-derived dendritic cells, are permissive for efficient proliferation. In contrast, macrophages, monocytes and neutrophils may represent an obstacle in the cycle of L. major proliferation, release and infection of new cells. Therefore, besides their well-described function for priming and activating T cell effector functions against L. major, monocyte-derived dendritic cells provide a reservoir for rapidly proliferating parasites that disseminate at the site of infection. Supported by funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (ImmProDynamics, grant agreement n° 714233) and the German Research Foundation DFG (MU 3744/2-1 and SF8584-201/2) to A.J.M.
POSTER PRESENTATIONS

P.D4.07.15
RAKNL improves macrophage-mediated immunity to Leishmania major infection
T. S. Rigoni, M. P. Cabral-Picon, A. A. Filardo, M. F. Lopes, G. A. Do Reis; Institute of Biophysics Carlos Chagas Filho, Rio de Janeiro, Brazil.

Macrophages are central effector cells in the immune response to Leishmania infection and their functional characteristics can be modulated by environmental stimuli. Whereas M1 macrophages (promoted by IFNγ and LPS) are associated with parasite control through the production of nitric oxide, M2 macrophages (promoted mainly by IL-4) are permissive to infection. Signals from the anatomical site in which macrophages are found can also control their tissue-specific functions. For example, receptor activator of nuclear factor κappa B ligand (RAKNL) is an important factor for macrophage differentiation to osteoclasts in the bones and also exerts regulation of the immune response in infections. However, the role of RAKNL in macrophage functions in parasitic infections is still unknown.

Here, we demonstrated that treatment of inflammatory macrophages of B6 mice with RAKNL and IFNγ increased nitric oxide (NO) production, as well as production of inflammatory cytokines (IL-12 and TNFα). In addition, we observed reduced expression of the M2 marker MGL-1 and increased IL-12 and NOS expression in macrophages treated with RAKNL only. Treatment with RAKNL and IFNγ also reduced parasite load in macrophages infected with L. major, in an INOS and reactive oxygen species (ROS)-dependent manner.

Together, these data suggest that RAKNL per se and/or in cooperation with IFNγ enhances the effector activity of macrophages, by inducing M1 macrophage phenotype and promoting parasite control via NO and ROS production.

P.D4.07.16
Cathelicidins have their own unique fingerprint for antimicrobial and immunomodulatory activity
M. R. Scheenstra, M. Coorens, A. van Dijk, E. J. Veldhuizen, H. P. Haagsman; University Utrecht, Utrecht, Netherlands.

Cathelicidins are short cationic peptides, which play a crucial role during the innate immune response upon infection. Due to the combination of a strong antimicrobial effect combined with immunomodulatory capacities, they are promising alternatives to traditional antibiotics. The human cathelicidin LL-37, chicken cathelicidin-2 (CATH-2) and porcine PMAP-36 show similar antibacterial activities against E. coli. However, transmission electron microscopy (TEM) indicated that the mechanism used by these cathelicidins to kill E. coli are highly divergent, ranging from disrupting the membrane to complete lysis of the bacterial membrane. The immunomodulatory capacities of the cathelicidins were tested by studying the in vitro responsiveness of murine RAW264.7 macrophage cells with LPS-induced macrophage activation using isothermal calorimetry (ITC), we were able to show that the mechanism of LPS inhibition differs greatly between the peptides. PMAP-36 is able to bind up to three LPS molecules, whereas CATH-2 and LL-37 bind only one LPS molecule. In addition, the binding of PMAP-36 and CATH-2 to LPS is very strong, whereas LL-37 only weakly binds LPS. In conclusion, although LL-37, CATH-2 and PMAP-36 are equally efficient in E. coli killing and inhibiting LPS-induced macrophage activation, their mode of action differ greatly. Understanding the inhibitory mechanisms of cathelicidins during LPS-induced immune activation could help avoid unwanted immune activation and sepsis.

P.D4.07.17
A single subcutaneous injection of chicken cathelicidin-2 in mice enhances the immune response against specific TLR ligands
M. R. Scheenstra, A. van Dijk, T. Cuperus, J. L. Tjeerdema-van Bokhoven, E. J. Veldhuizen, H. P. Haagsman; University Utrecht, Utrecht, Netherlands.

Cathelicidins are short cationic peptides, containing both antimicrobial and immune modulatory activities. Previously it was shown that in ovo administration of a Δ-analog of chicken cathelicidin-2 (D-CATH-2) has a protective effect against a respiratory E. coli infection after hatch. D-CATH-2 treated chickens had reduced mortality, morbidity and bacterial load 7 days post infection. Similarly, a subcutaneous injection in mice of a truncated analogue of D-CATH-2, (DC-2), resulted in a protective effect against infection. To determine the mode of action of DC-2, different concentrations were administered subcutaneously in a single dose in mice. Increased numbers of monocytes in the blood of mice receiving the highest dose (10 mg/kg) were observed for up to 7 days. In addition, ex vivo stimulation of total splenocytes showed an increased immune response for specific TLR-agonists 24 hours post-injection, especially for the lower doses of DC-2, (0.1 and 1 mg/kg). Bone marrow derived macrophages (BMDM), cultured 7 days after DC-2 treatment, showed an increased activation upon stimulation with lipoproteins, indicating a prolonged effect of DC-2, treatment. To show this was really due to DC-2, injection, BMDM of naïve mice were treated in vitro with DC-2, early in culture. Upon stimulation, the DC-2–treated BMDM showed a similar phenotype as the in vivo trained mice. In conclusion, this study showed that a single subcutaneous injection of DC-2, has a prolonged protective effect in both chickens and mice. This suggests a promising role for derivatives of CATH-2 as alternatives to traditional antibiotics.

P.D4.07.18
ADCC antibodies correlate with reduced infection in a household model of influenza transmission

Antibody Dependent Cellular cytotoxicity (ADCC), have been shown to be increased in older adults accounting for reduced H1N1 pandemic infection and risk of infection in a human challenge study, whilst also showing evidence for cross reactivity to avian viruses. Furthermore, human experimental challenge studies have shown a correlation with high ADCC titers and reduced risk of infection. Data on the protective role that baseline reactive responses play in acquisition of infection and the severity of infection from baseline levels prior to infection is scarce. Our study reports on the context of household acquired infection in the Hong Kong community, and baseline PBMC and serum samples were collected from households reporting an index case of infection. Contacts of infected subjects were recruited and monitored for acquisition of infection. The baseline ADCC influenza-specific responses of uninfected contacts was found to be higher in magnitude and avidity to multiple influenza proteins than infected contacts, indicating a protective baseline levels prior to infection is scarce. Our study reports on the context of household acquired infection in the Hong Kong community, and baseline PBMC and serum samples were collected from households reporting an index case of infection. Contacts of infected subjects were recruited and monitored for acquisition of infection. The baseline ADCC influenza-specific responses of uninfected contacts was found to be higher in magnitude and avidity to multiple influenza proteins than infected contacts, indicating a protective role of ADCC antibodies in the acquisition of influenza infection. Experiments are ongoing to assess the role of ADCC for the role of glycan effector expression for these responses. This study provides rare data on the context of community acquired influenza infection and the protective threshold of baseline immune responses for ADCC antibodies.

P.D4.07.19
Imaging Intracellular Pathogens Using Correlative Superresolution Electron Microscopy
S. I. van Kasteren; Leiden Institute of Chemistry, Leiden, Netherlands.

Intracellular pathogens can survive inside phagocytes, despite a powerful arsenal of anti-bacterial agents present in these cells. Understanding the interaction and survival mechanisms of these pathogens is therefore of utmost important to ensure the development of better antibiotics against these agents. To achieve this, we have developed a new imaging approach to study this interaction in detail: we can image pathogens selectively inside host cells using a technique called correlative light-electron microscopy, which overlaps a fluorescent image (e.g. originating from a fluorescent protein) onto an electron micrograph to place it in the ultrastructural context of the cell. We have combined this technique with biorthogonal pathogen labelling, which allows the labelling of the proteome of a pathogen with very small click-chemistry handles. This approach negates the use of isothermal calorimetry (ITC), we were able to show that the mechanism of LPS inhibition differs greatly between the peptides. PMAP-36 is able to bind up to three LPS molecules, whereas CATH-2 and LL-37 bind only one LPS molecule. In addition, the binding of PMAP-36 and CATH-2 to LPS is very strong, whereas LL-37 only weakly binds LPS. In conclusion, although LL-37, CATH-2 and PMAP-36 are equally efficient in E. coli killing and inhibiting LPS-induced macrophage activation, their mode of action differ greatly. Understanding the inhibitory mechanisms of cathelicidins during LPS-induced immune activation could help avoid unwanted immune activation and sepsis.

P.D4.08.01
Silent recruitment of TL4R and MD-2 to the Chlamydia trachomatis serovar D inclusion in epithelial cells of the human urogenital tract
S. Albrecht; Institute of Medical Biology and Hygiene, Mannheim, Germany.

Cells recognize invading microorganisms through pattern recognition receptors presented on their surface, resulting in the release of inflammatory cytokines. For example, the Toll-like receptor (TLR) family member TL4 in combination with MD-2 and CD14 has been identified as the principle signal transducer in lipopolysaccharide (LPS) recognition. In the past, the role of TL4R in Chlamydia recognition in epithelial cells of the urogenital tract has been controversially discussed. Whereas in rat prostate epithelial cells endogenous TL4R is recruited to the C. muridarum inclusion, the co-localization of FFP-labeled TL4R to the C. trachomatis serovar L2 inclusion in human cervix cancer cells could not be monitored.

With our studies of endogenous TL4R in human cervix cancer and bladder cancer cells infected with C. trachomatis serovar D we want to give this discussion a new turn.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

**POSTER PRESENTATIONS**

**_immunofluorescence studies of endogenous TLR4 and MD-2 in these cells revealed that TLR4 and MD-2 are recruited to the bacterium as early as 6 h p.i. At later time points (24-30 h p.i.), TLR4 and MD-2 are still associated with the bacterial cell wall. Additionally, we analyzed the localization of the intracellular TLR4 adapters MyD88, TIRAP and TRAM. We observed that MyD88 and TIRAP, but not TRAM, are recruited to the C. trachomatis inclusion. However, our data show that IL-6 and IL-8 production could not be induced by C. trachomatis infection. We assume that C. trachomatis recognition by TLR4/MD-2 does not lead to a pro-inflammatory signal and therefore remains silent.**

**P.D4.08.02**

**NLRP3 suppresses neutrophil-mediated innate immunity to histamin**


Neutrophils are an important first line of defense against invading microorganisms but their role in innate immunity to large pathogens such as parasitic helminths is becoming increasingly appreciated. Understanding the mechanism of how these cells are activated and regulated following helminth infections is unclear. We demonstrate that rapid neutrophil recruitment to the lung is important for innate immunity to the nematode **_Nippostrongylus brasiliensis_** and these responses are suppressed by the NLRP3 inflammasome. NLRP3 deficient mice displayed elevated recruitment of neutrophils to the lung and enhanced protective type 2 immunity including elevated IL-4 and IL-13 expression in the lung and goblet cell hyperplasia in the intestine. Co-culture of sort-purified neutrophils with _Nippostrongylus_ larvae resulted in killing of the parasite, potentially representing a mechanism of how these cells may provide protection against infection. Our findings suggest that neutrophils are important for regulating the early innate immune response to gastrointestinal helminth infections, suggesting that targeting NLRP3 may be a novel approach for limiting parasitic helminth health burdens.

**P.D4.08.03**

**An in vitro study on the recruitment and differentiation of blood monocytes by spleen macrophages leading to macrophage hyperplasia during malaria**


1Sanquin Research, Amsterdam, Netherlands, 2CNRS Station Biologique de Roscoff, Roscoff, France.

Malaria is still a global health and economic burden, responsible for 2 million deaths annually. **_Plasmodium falciparum_** is the most pathogenic Plasmodium species causing severe clinical symptoms like cerebral malaria, renal failure and lactic acidosis. The less understood pathology and main cause of infant mortality in endemic countries is severe malarial anemia (SMA). SMA is accompanied by splenomegaly and macrophage hyperplasia. Therefore, the elucidation of the mechanism facilitating the massive accumulation of phagocytes in the spleen could give an insight into the severe pathology of malarial anemia. By applying confocal microscopy and live cell imaging, we visualized the recruitment of circulating monocytes to spleen macrophages, which had been challenged with _P. falciparum_ infected red blood cells (iRBCs). Moreover, the recruited monocytes show a red pulp macrophage-like morphology characterized by the direct cell interaction with splenomegaly and the erythropagocytosis of iRBCs. Together these findings indicate the recruitment and accumulation of monocytes to the spleen upon malaria infection, thus, contributing to macrophage hyperplasia and the subsequent massive destruction of uninfected and infected red blood cells, ultimately leading to severe malarial anemia.

**P.D4.08.04**

**Respiratory epithelial cells enhance IFN-γ production by natural killer and mucosal associated invariant T cells in response to Bordetella pertussis**


IFN-γ is important for protective immunity to Bordetella pertussis, the causative bacterium for whooping cough. Here we identify an innate mucosal mechanism for the production of IFN-γ in the early stages of infection. PBMCs from healthy donors were stimulated with proteins or inactivated intact _B. pertussis_ (B1917) for 20h in the presence or absence of respiratory epithelial cells. Cell culture supernatants were analysed for cytokine production and cells for intracellular IFN-γ and CXCL10 production. Stimulation of PBMCs with multiple isolates of _B. pertussis_, but not soluble ligands of _B. pertussis_, and IL-15 resulted in synergistically increased secretion of IFN-γ. Intracellular staining revealed that epithelial cells were the main source of IFN-γ, but not MIP4 or CCR7 cells. Depletion of monocytes or pDCs did not impair but rather increased the levels of IFN-γ. Purification of untouched NK cells showed that NK cells can produce IFN-γ following exposure to _B. pertussis_ and IL-15 independent of other immune cells. Both levels of IFN-γ in the supernatant and intracellular IFN-γ increased when PBMCs were cultured in the presence of respiratory epithelial cells. Also, IFN-γ inducible chemokines CXCL9 and CXCL10 increased and staining showed that CXCL10 was mainly derived from epithelial cells. We conclude that respiratory epithelial cells enhance rapid IFN-γ production by NK and MAIT cells following stimulation with _B. pertussis_. These data provide insight into immune responses to _B. pertussis_ that could aid the development of therapeutic strategies.

**P.D4.08.05**

**Rhinovirus induces an anabolic reprogramming in host cell metabolism essential for viral replication**


Rhinoviruses (RVs) are responsible for the majority of upper airway infections; despite their high prevalence and the resulting economic burden, effective treatment is lacking. We report herein that RV induces metabolic alterations in host cells, which offers an efficient target for antiviral intervention. Metabolic analysis of RV infected cells revealed a critical redox deficiency. Glucose uptake is increased extracellularly and intracellularly; pools -via glycogenolysis- for viral replication. The virus-induced enhancement of glucose uptake was dependent on PI3-Kinase and was accompanied by the upregulation of GLUT1 surface expression. Glucose was primarily required to attain a highly anabolic state in the infected cells including the upregulation of essential anabolic enzymes. Collectively, we observed that glucose-deprivation both from medium and via glycolysis inhibition by 2-deoxyglucose (2-DG) potently impairs viral replication. Metabolic analysis showed that 2-DG specifically reverts the RV-induced anabolic reprogramming. In addition, treatment with 2-DG inhibited RV infection and inflammation in a murine model. Thus, we demonstrate that the specific metabolic fingerprint of RV infection can be used to identify new targets for therapeutic intervention.

**P.D4.08.06**

**The mitochondrial sirtuins SIRT3 and SIRT5 control NLRP3 inflammasome activation and interleukin-1β (IL-1β) secretion**

T. Heinonen, E. Carló*, D. Le Roy*, J. Auwerx*, T. Roger*; 1Louvain University Hospital, Eupenlges, Switzerland, 2Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

**Background:** IL-1β is involved in host defences against infections, but dysregulated expression of IL-1β is involved in inflammatory and metabolic disorders. The NLRP3 inflammasome, which is activated by reactive oxygen species (ROS), cleaves pro-IL-1β into mature, secreted, IL-1β. SIRT3 and SIRT5 are mitochondrial lysine deacetylases regulating the activity of ROS detoxifying enzymes. We previously showed that single knockout in SIRT3 or SIRT5 has no impact on innate immune responses. Hypothesizing that SIRT3 and SIRT5 may have compensatory activities, we assessed whether double deficiency in SIRT3 or SIRT5 has no impact on innate immune responses. Hypothesizing that SIRT3 and SIRT5 may have compensatory activities, we assessed whether double deficiency in SIRT3 or SIRT5 has no impact on innate immune responses. Hypothesizing that SIRT3 and SIRT5 may have compensatory activities, we assessed whether double deficiency in SIRT3 or SIRT5 has no impact on innate immune responses. Hypothesizing that SIRT3 and SIRT5 may have compensatory activities, we assessed whether double deficiency in SIRT3 or SIRT5 has no impact on innate immune responses. Hypothesizing that SIRT3 and SIRT5 may have compensatory activities, we assessed whether double deficiency in SIRT3 or SIRT5 has no impact on innate immune responses.

**Methods:** Mice were housed in SPF conditions. Bone marrow derived macrophages (BMDMs) were primed with TLR ligands and stimulated with monosodium urate (MSU) crystals to assess the production of ROS and cytokines. Mice were injected i.p. with MSU crystals and i.v. with listeria monocytogenes. Peritoneal lavage and blood were collected to quantify ROS, cytokines and bacteria. Weight, severity scores and survival were registered.

**Results:** SIRT5/s deficiency increased ROS production and IL-1β release by BMDMs, and ROS production by peritoneal cells. In a model of listeriosis, SIRT3/s deficiency was associated with reduced bacterial burdens, but had no significant effect on mouse survival.

**Conclusion:** These results suggest that SIRT3 and SIRT5 cooperate to control inflammasome activity and IL-1β production, and to protect from listeria burden. Thus, dual targeting of SIRT3 and SIRT5 may represent an attractive strategy for treating IL-1β-mediated inflammatory and metabolic diseases without increasing the risk of infection.

**P.D4.08.07**

**Metabolic re-programming of the innate antiviral response during dengue virus infection of myeloid dendritic cells**


Dengue virus (DENV), the leading arthropod-borne viral infection in the world, infects more than 300 million people worldwide, leading to 50,000 deaths annually. Markers associated with oxidative stress have been identified in patients with severe DENV infection, suggesting a relationship between oxidative stress and viral pathogenesis. Using genetic, biochemical and pharmacologic approaches, we demonstrated that the antioxidant gene network induced by Nrf2 transcription factor limited antiviral and cell death responses to DENV infection in primary human monocyte-derived dendritic cells (Mo-DC). Recent studies have further demonstrated that Nrf2, activated by the chemical sulfopharine (SFN) or by the Krebs cycle metabolite itaconate, dampened the release of pro-inflammatory cytokines, type I IFNs and IFN-stimulated genes, including the cGAS-STING, in response to DENV infection. Silencing of Nrf2 by RNA interference or CRISPR/Cas knockout increased both DENV infection and the associated antiviral and inflammatory responses.
As a viral evasion strategy, de novo DENV infection in turn targeted NR2f for proteasome-mediated degradation, and also down-regulated metabolic pathways involved in NADPH and ATP production, thus altering the reprogramming of the antioxidant response during DENV infection potentially establishes metabolic conditions for ROS accumulation and oxidative stress that aggravates DENV pathogenesis. Collectively, these data indicate that NR2f and the anti-oxidant gene network as important regulators of the innate antiviral and inflammatory response, and as a target for DENV-mediated metabolic re-programming of the host response to infection.

PD.4.08.07 Immune evasion strategies of HCMV: Functional characterization of the highly polymorphic HCMV Fcy receptor RL12/gp95
K. Hoffmann1, E. Mercé-Maldonado2, H. Reinhardt1, E. Corrales-Aguilar1, V. Khanh Le-Trilling1, P. Lacher3, H. Hengel3
1Institute of Virology, Freiburg, Germany, 2Institute for Virology, Heinrich-Heine-University, Düsseldorf, Germany, 3Virology-CIET, Faculty of Microbiology, San José, Costa Rica.

HDV virology

Interactions of IgG with Fcγ-Receptors (FcyRs), expressed on many immune cells, are essential for opsonization, phagocytosis and antibody-dependent cellular cytotoxicity. To avoid harmful IgG effecter responses HCMV has evolved evasion strategies by expressing viral FcyRs (FvγRs) interfering with host FcyR activation. HCMV encodes several, FvγRs, i.e. gp34 (RL11), gp68 (UL119-UL118) and gp95 (RL12).

For gp34 and gp68 we have demonstrated a powerful inhibition of all activating FcyRs, i.e. FcyRI/CD64, FcyRII/CD32A and FcyRI/CD16. Here we focused on RL12/gp95 as a further potential antagonist of host FcγRs in further to gp34 binding of gp95 and oxidative stress. Metabolic differences between different phenotypic pattern of gp95 subclass-expression while gp34 and gp68 bind readily to all human IgG subclasses, gp95 binding is restricted to human IgG1 and IgG3. Further differences were observed with respect to gp95 efficiency to antagonize human FcyRs. Using Rituximab (anti-CD20) as a subclass-specific dependent reference strong FcγR-like effects against FcyRI/CD64 > FcyRII/CD32A/FcyRI/CD16 were noted while only minor effects on FcyRI/CD64 were seen, contrasting gp68 which invariably blocked all human FcyRs with a comparable efficiency.

Unlike gp34, UL119-UL118, RL12 is one of the highly polymorphic HCMV-encoded FcγRs. Its sequence analysis revealed that most of the MHC class I downregulating strain is a gp95 (RL12) allele from a HCMV strain of the B5亚群, however the role of RL12 in HCMV pathogenesis is poorly understood. The alpha polypeptide (hRL12) is conserved across all HCMV strains, however the functional role of hRL12 in HCMV pathogenesis is, however poorly understood.

Results: hRL12 in transgenic mice is not able to alter virus replication, however results on the other hand offer hints about potential hRL12 interaction partners and reveal that hRL12 is ubiquitinated independent of host cell or parasite mediated interaction. Therefore, the aim of this study was to understand the mechanisms by which hRL12 is able to ubiquitinated independent of host cell or parasite regulated interaction. The results were confirmed in an in vivo model of HCMV infection in the mouse, i.e. the BALB/c mouse. In this model RL12 is able to reduce the rate of virus replication in vivo.

Conclusions: The results presented here indicate that hRL12 is able to ubiquitinated independent of host cell or parasite regulated interaction.

PD.4.08.10 IL-26 inhibits Hepatitis C virus replication in hepatocytes
V. Lorchette1, E. Beaumont1, L. Preissler2, P. Pigom3, S. Blanchard7, J. Davet3, M. M. Portaro1, A. Morel1,2, H. Fickenscher7, Y. Delhéstrez1,2, P. Roingeard7, J. Peulvast1,2,4,6
1Institute of Biochemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, 2Institute of Medical Microbiology and Hospital Hygiene, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany, 3Institute of Virology, Freiburg, Germany, 4Institute of Medical Microbiology and Hospital Hygiene, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany, 5Institute of Microbiology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany, 6Institute of Immunology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany, 7Institute of Pathology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany.

IL-26 is a proinflammatory cytokine which plays an essential role in innate host defense against microbial pathogens. Recently, IL-26 was also shown to protect against HCV infection in human hepatoma cells. Here, the antiviral activity of IL-26 was studied in primary human hepatocytes.

Materials and Methods: Primary human hepatocytes were obtained from deceased donors (n = 10) and were cultured for 7 days. Hepatitis C virus infection was carried out by transduction of cells with HCV-JFH1, a cell culture prototype of HCV genotype 2a. Cell viability and intracellular virus replication were measured by RT-qPCR and CPE.

Results: IL-26 protected primary human hepatocytes from HCV infection in a dose-dependent manner. In the presence of IL-26, intracellular HCV RNA levels were reduced by 80% while CPE was not observed.

Conclusions: IL-26 is a potent antiviral cytokine against HCV infection in primary human hepatocytes. These findings may have potential implications for the development of novel therapeutic strategies against HCV infection.

PD.4.08.11 mGBPs and interacting proteins in the combat against Toxoplasma gondii infection and emerging insights into the biochemical properties of mGBP7
L. Legewie1, S. Smits1, N. Steffen1, E. Kravets2, L. Schmitt2, K. Pfeffer1, A. Stum1, E. Mercé-Maldonado1, S. Kaufmann1, H. Fickenscher2, P. Roingeard7, J. Peulvast1,2,4,6, J. Weiner1
1Institute of Immunology, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany, 2Institute of Medical Microbiology and Hospital Hygiene, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany, 3Institute of Medical Microbiology and Hospital Hygiene, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany, 4Institute of Pathology, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany, 5Institute of Microbiology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, 6Institute of Immunology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, 7Institute of Biochemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany.

Introduction: Toxoplasma gondii (T. gondii) is an obligate intracellular parasite and the causative agent of toxoplasmosis. During host cell infection, T. gondii forms an unique membranous compartment called the parasitophorous vacuole (PV). Members of the murine guanylate binding protein (mGBP) family translocate to the PV and mediating its disruption, but underlying molecular mechanisms are poorly understood. The aim of this project is to provide a better understanding of the biochemical properties of mGBPs as well as to elucidate the molecular mechanisms by which this IFNγ-inducible protein family is able to attack the parasite directly.

Methods: The GTPass activity of mGBP7 was analysed using the malachite green phosphate assay and SEC-MALS. For the identification of potential mGBP7 interaction partners originating from the host cell or the invading T. gondii co-immunoprecipitation (co-IP) and mass spectrometry (MS) experiments were performed. Results: The GTPass activity of mGBP7 indicated an optimal concentration at 0.01 µM, a maximal concentration of 250 µM in different cell lines, i.e. HeLa in the absence of exogenous TNF-α and 250 µM in the presence of 250 µM. In the latter condition, the Hill coefficient the SEC-MALS results support the assumption that mGBP7 stimulates the GTPass activity in a cooperative manner and constitutes a very transient oligomer. The MS results on the other hand offer hints about potential mGBP7 interaction partners and reveal that mGBP7 is ubiquitinated independent of T. gondii infection.

Conclusions: The biochemical characterization of mGBP7 and the identification of potential mGBP7 interacting proteins offers new insights into the dynamic interactions at the interface of parasite and host.
C-type lectin receptor (CLR)-Fc fusion proteins as a tool to screen novel CLR/bacteria interactions
S. Mayeri, R. Moeller, J. Monteiro, K. Elliott1, C. Jasenhos2, B. Lepenies3

1Immunology Unit & Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine Hannover, Hannover, Germany; 2Institute for Medical Microbiology, Medical School Hannover, Hannover, Germany; 3German Center for Infection Research (DZIF), Partner site Hannover-Braunschweig, Germany.

The host is challenged every day by a huge number of various pathogens. Highly conserved microbial structures (pathogen-associated molecular patterns (PAMPs)) are recognised by pattern-recognition receptors (PRRs) localised on immune cells. Upon PAMP detection, several effector functions like phagocytosis and antigen presentation are induced. C-type lectin receptors (CLRs) represent one group of PRRs. While the interaction of CLRs with several pathogens-derived ligands has been described, still little is known about the role of CLRs in bacterial recognition.

This manuscript describes innovative methods based on a comprehensive library of recombinantly expressed CLR-Fc fusion proteins to unravel novel CLR-bacteria interactions. For demonstration, the important human pathogens Group A Streptococcus and Campylobacter jejuni were used for exemplary studies to demonstrate that these methods can be easily applied to Gram-positive as well as Gram-negative bacteria. First, a plate-bound ELISA-based assay was established to allow for a high-throughput pre-screening of potential bacteria-CLR interactions. Furthermore, a flow cytometry-based assay was used to screen for CLR-bacteria interactions in solution and finally, confocal microscopy allowed for visualization of CLR-bacteria characterisation of the interaction. Using this combination of different techniques, we have identified candidate CLRs that may play a role in bacterial recognition. Our study enables new insights into the host innate immune response against the respective bacteria.

Type I interferon induction by Orientia tsutsugamushi depends on nucleic acid recognition but does not require bacterial viability
Z. Orfanos1, V. Heftet1, S. Bauer1, C. Keller1, 2

1Institute of Virology, Philipp University Marburg, Marburg, Germany; 2German Centre for Infection Research at the Institute of Virology, Philipp University Marburg, Marburg, Germany.

Here we have identified a set of genes that are essential for colonization of mice lungs by high density transposon insertion site sequencing Tn-Seq on the whole genome of Group A Streptococcus. For demonstration, the important human pathogen Orientia tsutsugamushi, is a mite-borne zoonosis associated with strong cytoxine induction. Upon infection, Orientia enters phagocytic cells by receptor-mediated endocytosis and shortly after escapes the endosome to replicate in the cytoplasm. Infection results in a strong induction of TNF-a and type I interferon within a few hours. Previous work proposed that interferon (IFN)-β is exclusively induced by live bacteria in macrophages. However, ligands and receptors responsible for this induction remain unknown.

We are investigating type I interferon induction by live and in mouse bone marrow-derived dendritic cells (BMDC). Our experiments show that live organisms induce IFN-β mRNA in C57BL/6 BMDC within hours after infection. Stimulation of BMDC with bacteria inactivated at 95°C showed a dramatically reduced IFN-β induction. Surprisingly, bacteria inactivated at a lower temperature induced as much IFN-β as live bacteria. These results point to a heat-stable ligand that is differentially accessible to innate receptors depending on the viability or damage of the bacteria. The IFN-β induction was dependent on the endosomal Toll-like receptors 3, 7 and 9, suggesting that the ligand is a nucleic acid.

The nucleic acid induction in BMDC in response to Orientia does not require viable bacteria but can also be recapitulated by dead organisms under certain conditions. We propose that the accessibility of nucleic acid ligands to innate receptors is different in live, dead or damaged bacteria, and that their exposure in the endosome leads to distinct type I interferon responses.
POSTER PRESENTATIONS

P.D4.08.18
Human Epidermal Langerhans cells might constitute an underestimated HIV reservoir
S. Soluzze, J. Strab, N. Bayer, A. Rieger, V. Touzeau-Roemer, G. Stingl, G. Stary;
Medical University of Vienna, Vienna, Austria.

Introduction: Viral reservoirs are major obstacle to HIV eradication. The skin is a highly immunologically active organ and containing CD4+ T cells and Langerhans cells (LCs), the major targets of HIV infection. LCs can restrict HIV viral replication and are thought to be determinant in reducing the risk of HIV transmission at mucosal level. However, recent evidence suggests that also antigen presenting cells (APCs) could remain latently infected with HIV upon establishment of a chronic infection. Our project aims at understanding if the skin resident cells could harbor latently infected cells and eventually provide new insights in the mechanisms of HIV latency.

Methods: We collected skin biopsy from individuals during the viremic phase of HIV infection as well as under antiretroviral therapy (ART) with undetectable viral load (ndVL). We investigated HIV latency analyzing p24 expression by Tissue-FACS software and electron microscopy (EM).

Results: IF staining showed the presence of a CD4+ CD38+ epidermal immune cell population still harboring p24 expression both in viremic patients and those with ndVL. We identified this population as being exclusively composed by LCs.

Conclusions and future prospects: Although preliminary, our results suggest that LCs might still harbor HIV viruses in individuals with suppressed viral replication. Further analysis will prove if p24+ cells contain HIV RNA and DNA by RT-PCR as well as RNA and DNA Scope analysis and electron microscopy. If this is the case, our experiments will focus in understanding infected LCs could present replication competent virus and constitute an underestimated HIV reservoir.

P.D4.08.19
The Use of CRISPR-Cas9 Based Genome-Scale Screening for Mapping the Intracellular Immune Response of NK Cells Against Lentiviral Gene Delivery
1Inserm UMR 1232, NANTES, France, 2UMR 1089, NANTES, France, 3Institute of clinical medicine, Kuopio, Finland, 4UMR 1232, NANTES, France.

Pre-existing AAV8 CD8+ T cells are present in all donors and harbor a cytotoxic function.

1Inserm UMR 1089, NANTES, France, 2UMR 1232, NANTES, France.

Recombinant adeno-associated virus (rAAV) are the most widely used viral vector for in vivo gene therapy. Despite promising results in preclinical and clinical studies, pre-existing immunity against the viral capsid remains a major hurdle to the efficacy and safety of AAV-based gene transfer. Particularly, pre-existing anti-AAV CD8+ T lymphocytes can hamper gene transfer but their impact remains poorly defined.

In our study, we previously reported the use of Tetramer-Associated Magnetic Enrichment (TAME) to analyze frequency and phenotype of AAV-specific CD8+ T cells by flow cytometry in order to set up a more sensitive and comprehensive method to detect and characterize these cells. Tetramers loaded with several AAV peptides allowed detection of AAVB capsid-specific CD8+ T cells among PBMCs, without amplification, in all healthy HLA-A2/B7+ donors tested even in absence of anti-AAV IFN-γ ELISPOT positive response. Moreover, phenotypic assessment of the detected cells revealed a Th1 profile. To be more restrictive, we tested TAME with the immunodominant peptides described since. We also detected AAV8 capsid-specific CD8+ T cells in all tested donors but at lower frequencies. To characterize the impact of this TAA+ cells, we sorted AAV8-specific CD8+ T cells after TAME and we succeeded in generating AAV-specific CD8+ T cell lines that were cytotoxic when faced with AAV8-loaded target cells.

The dissimilarities observed between presence of AAV-specific CD8+ T cells and IFN-γ responses, highlight the need to understand the onset of pre-existing anti-AAV immunity on rAAV-based gene transfer and its impact on clinical outcome in order to develop optimal strategies.

Abs. 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Abstracts of the 5
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P.D4.09.01
Comparison of Techniques Measuring Cellular Reactive Oxygen Species in Circulating and Tissue Rat Neutrophils
N. Fazal, M. Baig; Chicago State University, Chicago, United States.

Objectives: Production of ROS is considered as an important neutrophil function. Neutrophils utilize oxygen radicals to kill phagoygosed bacteria as well as remodeling endothelial and epithelial tissues. Neutrophils have an important role to play both in innate immune response as well as tissue-remodeling and tissue repair. There are many known techniques to ascertain production and release of ROS in cultured cells, especially neutrophils. In this study we compared the efficacy and use of different methods and determined the kinetics of intracellular and extracellular oxidants production and/or release.

Methods: We used fluorometry to determine hydrogen peroxide, Photometry to measure superoxide dismutase inhibitable reduction of Cytochrome C and Luminometry to gauge peroxidase-dependent chemiluminescence namely (Isoluminol, Luminol and Lucigenin). We used rat neutrophils to obtain blood (circulatory) and peritoneal (tissue) to study both activated, PMA- or FMLP- or LPS-stimulated and /or un-activated cells.

Results: Our results show that all the techniques used in this study were able to measure both oxidant production as well as oxidant release in neutrophil cell culture assays. Kinetics of ROS production in studied Neutrophils show nice curves over 60-minutes assay times. Intraocular vs. Extracellular production/release of ROS were followed over every 5
5 seconds and different patterns of peak values, which were considerably significant, which were considered statistically significant.

Conclusions: Our results showed a kinetic cell culture assay that established base-line values of reactive oxygen species production and release, over first hour of neutrophil / cell stimulation with PMA and/or FMLP and/or LPS. These assays could determine the efficacy of therapeutic agents.

P.D4.09.02
Cutibacterium acnes plays a role in the maintenance of skin barrier and homeostasis
B. S. Bolla, L. Enderi, G. Tax, E. Urbain, L. Kemény1, K. Szabó1; 1Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary, 2Institute of Clinical Microbiology, University of Szeged, Hungary, Szeged, Hungary, 3MTA- Szte Dermatological Research Group, Szeged, Hungary, Szeged, Hungary.

Our skin provides a physical barrier to separate our body from the external environment. Little is known how the cutaneous microbiota may affect these functions, so our aim is to investigate the role of Cutibacterium acnes (C. acnes), member of the cutaneous microbiome using an in vitro cultured human keratinocyte cell line (HPV-KER) and 3D organotypic skin model systems (OS). Calcium-concentrated different cultur HPK-REF cultures were treated with C. acnes. Barrier changes were monitored measuring transepidermal electrical resistance (TEER), performing an xCELLigence analysis and lucifer yellow (LY) penetration assays. We also analysed the expression changes of tight junction (TJ) proteins (CLDN1, 4, OCLN and ZO-1) by western blotting and immunohistochemical staining. In the presence of high dose C. acnes bacterium the barrier properties deteriorated: TEER and cell index (CI) values gradually decreased, while parallel to that LY penetration increased at 24 and 72 hours after bacterial treatment in the HPV-KER cultures. Dye penetration was also enhanced in the OS models upon C. acnes treatment. The level and distribution of TJ proteins also changed, OCLN and ZO-1 increased and CLDN1 decreased after treatment in the monolayer cultures, which was similar to the changes in the differentiated, granular layer of the OS models. We hypothesize that C. acnes may actively modify our properties of the epidermal barrier by changing the expression and localization of certain TJ proteins and through this it can play a role in the maintenance of cutaneous homeostasis.

P.D4.09.03
A role for miRNA-mRNA interactions in host immunomodulation during controlled human hookworm infection

Hookworms have evolved to modulate the human immune response. Recent reports have demonstrated that helminth therapy has a beneficial effect in treating inflammatory diseases. Neutrophils are key cell type to host an anti-inflammatory environment and to promote healing of the host. The role of host helminth's miRNA in influencing host inflammatory response is poorly understood. Here we show that larval hookworm infection of C. elegans induces the upregulation of a potent host miRNA, mnt-17as, which targets ChiP-1 and regulates neutrophil immune response.

Materials and Methods: SiRNA knockdown of the transgenic phenotype and overexpression of the ChiP-1 transcript and mnt-17as were performed. We compared the inflammatory response of the Naaienhart x C. elegans double transgenic strain infected with either the wild type or mnt-17as deficient hookworms.

Results: We observed a significant increase in the inflammatory response in the mnt-17as deficient strain. Furthermore we show that mnt-17as is able to repress the transcription of ChiP-1 and therefore regulate host inflammatory response to the larval parasite.

Conclusions: MiRNA-based therapies for inflammatory diseases are emerging, but little is known about the role of helminth's miRNA's in host inflammatory response. Further understanding of the role of hookworm infection on host miRNA expression and function is needed to develop effective treatments.

P.D4.09.04
Adult and ADAM17 control of skin dendritic cell function
N. Diener, R. Backer1, S. Papoianono1, K. Dietzel-Schonberger1, E. von Stebut2, B. E. Clauser3; 1Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, 55131 Mainz, Germany, 2Department of Dermatology, University Medical Center of the Johannes Gutenberg-University Mainz, 55131 Mainz, Germany, 3Department of Dermatology, University of Cologne, 50937 Cologne, Germany.

Dendritic cells (DC) are strategically positioned at epithelial borders to the environment like the skin and are crucial modulators of immune responses. The ‘a disintegrin and metalloproteinase’ (ADAM) family of surface-expressed ectodomain-shedding proteases regulate multiple cell functions such as cell adhesion, migration or cytokine release/ signaling. Dysregulated shedding by ADAM10 and ADAM17 is critical for the development of different immune-mediated diseases. Both sheddases are expressed on DC in the skin and skin-draining lymph nodes (sDLN). To investigate their role in DC, we generated conditional knockout mice of either one of these proteases in all CD11c+ cells (ADAM10-KO and ADAM17-KO mice).

Analysis by flow cytometry revealed reduced DC numbers in the skin of ADAM10-KO mice, whereas DC homeostasis in ADAM17-KO mice was unchanged compared to control mice. Although DC migration to the sDLN was not altered under steady-state conditions and following FITC-painting in ADAM10-KO and ADAM17-KO mice in vivo, it was significantly decreased in skin explants in vitro. Moreover, while the phenotypic maturation of bone marrow-derived DC (BMDC) was only impaired in the absence of ADAM17, both ADAM10- and ADAM17-deficient BMDC secreted reduced levels of TNF-a upon TLR stimulation. Intriguingly, after physiologic low-dose infection with Leishmania major, ADAM17-KO mice developed significantly larger, persisting skin lesions with increased parasite burdens as compared to controls with self-healing lesions. In conclusion, these data demonstrate that ADAM10 and ADAM17 regulate DC homeostasis and maturation/activation. In ongoing experiments we are dissecting the mechanism of ADAM17 governing skin DC function during cutaneous leishmaniasis.

P.D4.09.05
Characterisation of Schistosoma mansoni Larval Extracellular Vesicle protein 1 (SmLEV1) an immunogenic, schistosome-specific protein, exhibiting developmentally regulated alternate splicing
T. A. Gasan1, S. Wilson1, J. Wawrzyniak, E. M. Tukahebwa2, J. Wawrzyniak1, I. W. Chalmers2, B. S. Bolla3, L. Kemény1, 1Department of Dermatology, University of Cologne, 50937 Cologne, Germany, 2Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, 55131 Mainz, Germany, 3Department of Dermatology, University of Szeged, Szeged, Hungary, 4IBERS, Aberystwyth, United Kingdom, 5Cambridge University, Cambridge, United Kingdom, 6University of Health, Kampala, Uganda.

An integral component of cellular communication, Extracellular Vesicles (EV) have been described in protozoa and metazoan parasites. Both larval and adult Schistosoma mansoni worms release pre-packaged EVs, but to what end? Characterising proteins within schistosome EVs will aid in discerning their function(s) and may help develop schistosomiasis control strategies. Therefore, this project aims to characterise the most abundant EV protein in the tissue-migrating schistosomula stage - Schistosoma mansoni Larval Extracellular Vesicle protein (SmLEV1). Comparative sequence analysis demonstrates that SmLEV1 has orthologs in all published Schistosoma genomes, but not outside of the genus, nor has any characterised protein domains. By employing qRTPCR, we discovered differential expression of SmLEV1 across the schistosome lifecycle, with peak expression in cercariae as well as male-biased expression in sexually-reproductive adults. Importantly, SmLEV1 exhibits developmentally regulated alternate splicing during infection of the mammalian host. Cercariea displayed a significantly different population of isoforms, with over twice the level of exon-5 expression, compared with adult worms, but only two-thirds the expression of exon-8. Recombinant expression of SmLEV1, has enabled investigation of the host’s response to SmLEV1, in the mouse model and endemic human populations.

500
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
Interestingly, preliminary serological analysis from S. mansoni infected individuals shows a strong IgG1 response against SmLEV1 with minimal antigen-specific IgG and IgE; this finding is congruent to antibody responses generated against other surface/released schistosome proteins. Collectively, these results highlight SmLEV1 as an abundant, novel schistosome-specific, EV protein. Finally a mouse vaccination trial has been conducted to investigate the potential protective capabilities of an SmLEV1 vaccine.

P.D.4.09.06
Co-inhibitory receptors expression on CD8+ T cells during T. cruzi infection
R. Grote-Gálvez, Y. Arana, T. Jacobs; Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Trypanosoma cruzi is an obligate intracellular protozoan parasite that in 30% of cases causes Chagas disease. Chagas disease remains the most important neglected vector-borne disease in Latin America affecting more than 10 Million people. Although an initial CD8+ T cell mediated immune response controls parasite replication successfully, a complete clearance fails, which leads to chronic infection. To address this question, we established mouse models based on infection of C57BL/6 mice with different T. cruzi strains. We found that during acute infection with T. cruzi the T cell compartment is modulated by transient induction of different co-inhibitory molecules. Tim-3 was most significantly induced and this upregulation correlated with a reduced TNFα production. Using different blocking strategies, we explored Tim-3 function during infection in vivo. The blockade of Tim-3 restored CD8+ T cell function and reduced parasitic reservoirs in the tissue. We also found that Tim-3 ligands were strongly expressed on cells from the myeloid compartment. Accordingly, Tim-3 was significantly induced in infiltrating myeloid cells and the parasite burden was reduced upon pharmacological inhibition of Tim-3. Our results also indicate the potential role of Tim-3 in the dynamic regulation of immune responses in chronic T. cruzi infection.

P.D.4.09.07
Helminth-induced interference with bystander immune responses and the role of type 1 regulatory T cells
W. Hartmann, M. Brunn, M. Breloer; Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Helminths are large multicellular parasites that infect approximately one third of the human population. To prolong their survival, helminths manipulate the immune response of their hosts. Thereby, not only helminth-specific but also non-helminth-specific bystander immune responses such as the immune response to a vaccine are suppressed. We have previously shown that infection of mice with the filarial nematode Litomosoides sigmodontis leads to systemic suppression of IgG responses to thymus-dependent model antigens. This finding is congruent to what was previously visible after clearance of L. sigmodontis infection indicating that immunosuppression, once established, is preserved independent of the presence of living helminth-parasites. We now demonstrate the relevance of this helminth-induced interference with bystander immune responses and performed vaccinations in a commercially available anti-influenza vaccine: Reduced hemagglutinin-specific Ig responses were linked with an impaired protection against influenza H1N1 A/Hamburg/NY1580/09 challenge. Mechanistically, B cell function was suppressed indirectly, via accessory follicular T helper cells (TFH). Likewise proliferation of adaptively transferred ovalbumin-specific CD4+ T cells was suppressed in L. sigmodontis infected mice, reiterating suppressed TFH expansion. To analyse the mechanism we performed flow cytometry cluster analysis. Foxp3+ regulatory T cells increased locally, but not systemically and were dispensable for helminth-induced suppression of bystander immune responses. By contrast, we observed a sustained local and systemic expansion of type 1 regulatory T cells expressing Lag-3 and CD49b in helminth-infected mice and those with a history of helminth infection. We are currently characterizing the role of Foxp3 cells as potential mediators of suppression.

P.D.4.09.08
Myobacterial Growth Inhibition is associated with trained innate immunity
S. A. Joosten1, K. van Meijgaardens2, S. M. Arend1, C. Prijs1, F. Oftung1, G. Karsuva1, S. V. Kuk1, R. J. Arts2, R. van Crevel1, M. G. Netea1, T. H. Ottenhoff1; 1Leiden University Medical Center, Leiden, Netherlands, 2Norwegian Institute of Public Health, Oslo, Norway, 4KNCV Tuberculosis Foundation, The Hague, Netherlands, 5Radboud University Medical Center, Nijmegen, Netherlands.

The lack of defined correlates of protection hampers development of vaccines against tuberculosis (TB). In vitro mycobacterial outgrowth assays are thought to better capture the complexity of the human host/mycobacterium tuberculosis (Mtbs) interaction. Here, we used a PBMC-based “mycobacterial-growth-inhibition-assay” (MGI) to investigate the capacity to control outgrowth of Bacille Calmette-Guérin (BCG). Interestingly, strong control of BCG outgrowth was observed almost exclusively in individuals with recent exposure to Mtbs, but not in (long-term) latent TB infection, and only modestly in BCG vaccines. Mechanistically, control of mycobacterial outgrowth strongly correlated with the presence of a CD141+ monocyte population, but also required the presence of T-cells. The non-classical monocytes produced CXCL10, and CXCL10 receptor blockade inhibited the capacity to control BCG outgrowth. Expression of CXCR3 splice variants was altered in recently Mtbs exposed individuals. Since we observed strong MGI control recently after Mtbs exposure and we found a strong association with monocytic cells we hypothesized that trained innate immunity was responsible for the observed MGI control. Indeed, cytokines previously associated with trained immunity were detected in Mtbs exposed individuals. These data indicate that CXCR3-ligands are associated with trained immunity and critical factors in controlling mycobacterial outgrowth. In conclusion, control of mycobacterial outgrowth early after exposure to Mtbs is the result of trained immunity mediated by a CXCL10-producing non-classical CD141+ monocyte subset.

P.D.4.09.09
Enhanced cancer immunosurveillance by viral infection and Toll-like receptor ligation
M. F. M. Doudou1,2, J. P. Coutelier1; 1Dedicated institute - UCL, Brussels, Belgium, 2Department of Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Infection is a strong stage of cancer development, appropriate immune surveillance eliminates most of the transformed cells. In addition to cytotoxic T cells, innate immune cells such as natural killer cells and NK/T cells play a major role in this protection against cancer development, especially through production of interferon-gamma (IFN-γ). Since infections deeply modulate the immune microenvironment and particularly its innate components, we investigated their role in cancer immunosurveillance. Materials and Methods: The effect of infections on plasmacytoma [TEPC103.32] and mesothelioma (A81) cell growth was analysed in BALB/c mice after infection with lactate dehydrogenase-elevating virus (LDEV), a usually non-pathogenic mouse nidovirus. Infections were also mimicked by ligation of various Toll-like receptors (TLRs). The mechanisms of immunosurveillance were analysed by using anti-NK cell depleting polyclonal antibody and cytokine neutralizing monoclonal antibodies. Results: Acutely infected animals were significantly protected against both plasmacytoma and mesothelioma development. The protection was mediated by NK cell activation, through IFN-γ production. In addition, TLR-3, 7 and 9 ligation significantly protected mice against mesothelioma, but not plasmacytoma development. Conclusions: Our results indicate that modulation of the mouse immune microenvironment, and especially of innate immune responses, following either a non-pathogenic viral infection or a TLR ligation protects against mesothelioma and plasmacytoma early development.

P.D.4.09.10
Hypoxia-inducible factor-1 alpha deficiency results in dysregulated lipid metabolism associated with increased susceptibility toLeishmania donovani infection
I.Mesquita1, C. Ferreira1, D. Moreira1, G. Kluck1,2, A. Barbosa1, E. Torrado1, R. Dinis-Oliveira1,2, F. Rodrigues1,2, C. Cunha1,2, A. Carvalho1,2, A. Castro1,2, J. Estaquier1,2, R. Silvestre1; 1Life and Health Sciences Research Institute (ICVS), School of Medicine, Braga, Portugal, 2ICVS/3B's–PT Government Associate Laboratory, Braga, Portugal.

Leishmania donovani is an obligate intracellular protozoan parasite associated with the human disease leishmaniosis. It exploits these pathways to alter parasite specific CD8+ T cell responses and the role of type 1 regulatory T cells. Our results are an important contribution towards the multiple understanding of the immune evasion strategies of T. cruzi and identification of potential targets for novel immunotherapies.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - The Netherlands

P.D4.09.11

miR-29, miR-425, miR-15A and miR-126 were up-regulated. miR-29 is known to be present in HPV patients blood and to induce the PRRSV viral replication. ZIKV was also very little is known about the involvement of

P.D4.09.12

P.D4.09.13

In the current study, we demonstrate that while viral vector entry to NK cells can take place without major problems, the activation of antiviral signaling pathways leads to the

P.D4.09.14

LPCAT2 knockdown influences both Rough and Smooth Lipopolysaccharide-induced Toll-Like Receptor 4 Signalling in RAW264.7 Macrophages.

P.D4.09.15

Evaluation of miRNAs role in the immunopathogenesis of microcephaly caused by ZIKV in experimental models

P.D4.09.16

Neuroimmune Interactions Laboratory - Department of Immunology - University of São Paulo, São Paulo, Brazil, 1Neuromucosal Interactions Laboratory - Department of Immunology - University of São Paulo, São Paulo, Brazil.

P.D4.09.17

miRNAs during ZIKV experimental infection. We performed

P.D4.09.18

LPCAT2 knockdown might be affecting the MyD88-dependent TLR4 signalling pathway more than the MyD88-independent pathway. LPCAT2 plays a role in TLR4 signalling which is important in innate immunity. Therefore, LPCAT2 can be an effective target for anti-inflammatory treatments.

P.D4.09.19

Viral infections have always been the cause of serious human diseases, usually increasing rates of morbidity and mortality worldwide. Recently, the flavivirus Zika virus (ZIKV) was introduced especially in Brazil, causing alarming increase in the number of babies born with microcephaly. The expression of Interferon stimulated genes (ISGs) is very important in blocking viral replication during disease, and they may be modulated by several different factors through post-transcriptional mechanisms, in which, miRNAs play a key role. Still very little is known about the involvement of miRNAs during ZIKV infection. In this context, we evaluated the role of miRNAs during ZIKV experimental infection. We performed miRNAs analysis in vitro using cells of the central nervous system and, in vivo, with S.I animals susceptible to infection. Analyzing human neuronal precursor cells we observed that miR-29, miR-425, miR-15A and miR-226 are up-regulated. miR-226 is known to be present in HIV patients blood and miR-226 and to induce the PRRSV viral replication. ZIKV was also able to down-regulate 19 different miRNAs, among them, miR-9, known to increase mesenchymal stem cells differentiation towards neuronal cells, being an important factor for neurogenesis. In S.I fetal brain, we observed nine miRNA up-regulated and only miR-32 was down-regulated. Taken together, these data are indicative that ZIKV is able to modulate miRNA profile, evidencing the importance of these regulatory molecules, which may help us to better understand immunopathogenic mechanisms of microcephaly caused by ZIKV.
Poster Presentations

PD.04.09.16 Site-specific effects of IL-33 treatment during helminth infection

M. Reitz, N. Rüdiger, M. Brunn, M. Brodeer;
Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Strangylodes ratti is a rodent specific parasitic nematode that displays tissue migrating and intestinal life stages. Infective larvae actively penetrate the skin of their host, migrate within 2 days via the skin and lung to the mouth. They are swallowed, reach the intestine and moult to adults that reproduce by day 5. Infected mice terminate the infection in the context of a type II immune response. Thereby infection-induced expression of the alarmin IL-33 by alveolar epithelial cells was shown to promote the type II response in the lung and was associated with efficient expulsion of S. venezuelensis from the intestine. Here, we intend to dissect IL-33 mediated effects on the immune response to Strangylodes infection in the tissue and the small intestine. S. ratti infected mice showed a drastic reduction of parasitic adults in the intestine on day 6 after previous intranasal and intraperitoneal treatment with recombinant IL-33 (rIL-33). The reduced parasite burden correlated with increased activation of mast cells that are central for expulsion of S. ratti from the intestine. In contrast, no IL-33 administration after the tissue migration phase reduced intestinal parasite burden. In contrast, numbers of migrating larvae in skin, lung and head were significantly increased in rIL-33 treated mice indicating that the reduced intestinal parasite burden is not due to an improved immunity in the tissue. In summary, our data show that IL-33 displays contradictory and site specific effects on tissue migrating and intestinal S. ratti parasites. We are currently investigating the underlying mechanism.

PD.04.09.17 Malaria-induced FOXP3 regulatory T cells in disease progression and memory establishment

M. Richn, M. S. Mackroth, A. Abel, C. Steep, T. Jacobs;
Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Malaria is a major burden on global health with approximately 1200 deaths per day. After infection, immunity has to be strictly regulated to control the parasite but also to avoid immune pathology. Interestingly, in malaria experienced patients protection against severe malaria develops faster than protection against parasitemia. Recently, we could show that acute malaria induces CD4+PD1+Foxp3 T cells in humans. These cells can suppress effector T cells, which may contribute to reduced immune pathology but also may suppress protective immunity.

We could confirm induction of these suppressive cells by blood stage infection employing the P. berghei ANKA mouse model. High numbers of these Treg-like cells correlated with reduced immune pathology during reinfection, whereas parasitemia is not altered in comparison to the initial infection. By combining flow cytometry and T-Distributed Stochastic Neighbor Embedding (t-SNE), we could delineate a unique phenotype of these malaria-induced T cells characterized by a high expression of multiple co-inhibitory molecules. The highest amount of these Treg-like cells was found in the liver compared to spleen. Furthermore these cells exhibit different phenotypes in liver and spleen with regard to the expression of co-inhibitory molecules. This indicates an organ-specific component implicated in the induction of malaria-specific T-reg cells. Taken together, we could show that malaria-induced Treg cells have an important immune regulatory function in mice and humans and should be considered in malaria therapy and vaccination. Moreover, the accumulation of Treg cells in the liver highlights the unique tolerogenic environment of this organ and the influence on disease progression

PD.04.09.18 Batf3 deficient mice are protected against experimental cerebral malaria due to successful immune regulation

1Institute of Medical Microbiology, Immunology and Parasitology, University of Bonn, Bonn, Germany; 2Centre for Infectious Diseases, Parasitology Unit, Heidelberg, Germany.

Batf3 is a key component of the c-Jun NH2-terminal kinase pathway. Batf3-/- mice are hypersusceptible to T. gondii and T. cruzi infection, indicating a role for Batf3 in antiparasitic immunity. To further explore the role of Batf3 in antiparasitic innate immunity, we performed an in vivo screen to identify novel natural products with anti-T. gondii activity. A total of 300 new natural products and 30 derivatives thereof were analysed against T. gondii and multidrug resistant gram-negative rods (4MRGN).

Results: Within a first round of screening, promising candidates could be detected. This project will hopefully identify new anti-microbial products for novel therapies of infectious diseases.

Materials and Methods: Screening of natural products, which are able to inhibit Toxoplasma proliferation and MTT assays are performed. To identify anti-4MRGN products microdilution assays are performed.

Conclusions: This project will be the identification of novel natural products with anti-microbial activities against T. gondii and multidrug resistant gram-negative rods (4MRGN)

PD.04.09.19 Identification of new natural products with anti-microbial activity against Apicomplexa and multiresistant gram-negative rods (4MRGN)

S. Shanne Sazzadeh, S. Schmidt, M. Brunn, M. Breloer; 1Institute of Pharmaceutical Biology and Biotechnology, Duesseldorf, Germany.

Introduction: Anti-microbial therapies have successfully treated infectious diseases. However, the recent occurrence of (multi-) resistant pathogens increases lethality and morbidity of infected patients. Apicomplexa also develop resistance against established treatments. Therefore the need for new anti-microbial drugs is urgent. The primary aim of this project will be the identification of novel natural products with anti-microbial activities against Toxoplasma gondii and multidrug resistant gram-negative rod-shaped bacteria (4MRGN). The selection of targets is based on the elucidation of their targets for development of new leads for anti-microbial therapies.

Materials and Methods: Screening of natural products, which are able to inhibit T. gondii proliferation without being cytotoxic against HFF (human foreskin fibroblasts). This is accomplished via Toxoplasma proliferation and MTT assays. To identify anti-4MRGN products microdilution assays are performed.

Results: Within a first round of screening, promising candidates could be detected. This project will hopefully identify new anti-microbial products for novel therapies of Apicomplexa and multiresistant pathogens. Furthermore, after performing the MTT assay none of the natural products demonstrate cytotoxicity against both cell lines, which were used in Toxoplasma proliferation assay except Bionectriamidea A.

Conclusions: A total of 300 new natural products and 30 derivatives thereof were analysed against T.gondii (type I, BK strain and also type II, ME49 strain). Eleven products demonstrated anti-Toxoplasma activity and have been selected for further analyses.

PD.04.09.20 Lymphotoxin β receptor: A crucial role in Toxoplasma gondii infection

A. Wichert, U. R. Sorg, A. Degrando, K. Pfeffer;
Institute of Medical Microbiology and Hospital Hygiene, Düsseldorf, Germany.

Introduction: After infection with the obligate intracellular protozoan parasite Toxoplasma gondii (T. gondii) the production of cytokines induces potent cell autonomous effector mechanisms which can inactive the pathogen. Lymphotoxin β receptor (LTBR) signalling plays an important role in efficient initiation of innate and adaptive host responses to a variety of pathogens. The up-regulation of the murine 65kDa guanylate-binding proteins (mGBPs) via interferon γ receptor (IFNGR) signalling plays an essential role in survival of mice infected with T. gondii. mGBPs recruit towards the parasitophorous vacuole (PV) encapsulating the parasite, leading to the disruption of the PV and subsequent killing of the parasite. Compared to wildtype mice, LTBR deficient (LTBR-/-) mice show a markedly increased mortality and delayed up-regulation of mGBP expression in the acute phase of T. gondii infection. Methods: Immune responses (particularly B and T cell responses) as well as mGBP localization and function in LTβR-/- mice were investigated in vivo and in vitro experiments. Results: Analysis of IgM and IgG antibody responses suggests defects in Ig-class switching in LTBR-/- mice while FACS analysis of immune cell populations demonstrates that these mice are generally able to generate T. gondii specific CD8+ T cells. Initial in vitro experiments demonstrate that after IFNγ stimulation mGBPs in LTBR-/- fibroblasts are able to locate to the PV. Conclusion: These data suggest that defects in IFNγ mediated mGBP upregulation as well as dysfunctional Ig-class switching may contribute to the decreased survival rates of LTBR-/- mice.

PD.04.09.21 Host-Targeted Therapeutic Immune Protection against Anthrax

M. Zeng, Y. Yan1, H. Wang1, Y. Chen1, Z. Zheng2, H. Yang1, S. Francois1;
1Center of Emphasis in Infectious Diseases, Texas Tech University Health Sciences Center El Paso, El Paso, United States, 2Key Laboratory of Oral Medicine, Guangzhou Institute of Craniomaxillofacial Disease, Stomatology Medical Hospital of Guangzhou University, Guangzhou, China.

Introduction: To combat emerging and re-emerging infectious disease, it is more cost effective to use therapeutic vaccines for postexposure treatment than mass vaccination with preventive vaccines. Anthrax is an infectious disease caused by Bacillus anthracis that can secrete anthrax toxins including protective antigen (PA), lethal factor (LF), and edema factor (EF). Previously, we have shown that RNA inhibition of anthrax toxin receptors (TEMBV and CMG2) was protective against the cytotoxicity of anthrax toxins. However, inefficient cytokine delivery and toxicity of siRNA delivery vehicles limit the use of siRNA as therapeutics.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 503
Materials and Methods: In this study, we have developed a detoxified anthrax edema toxin, which consists of PA and nontoxic N-terminal fragment of EF (EFn) conjugated with a peptide nona-D-arginine residues (EFn-9dR) to enable siRNA binding. The detoxified toxin-siRNA was used to treat cells and C57BL6/j mice and evaluate if they can be protected against anthrax.

Results: The detoxified toxin complex was able to deliver specific siRNA to induce cmg2 gene silencing in different cell lines and C57BL6/j mice, and provide significant protection against anthrax lethal toxin challenge. Survived mice from toxin challenge were fully protected against lethal challenge with B. anthracis Sterne spores. The immune protective mechanism is mainly due to the significantly high serum neutralising antibody response against anthrax toxins in the mice.

Conclusions: The detoxified anthrax toxin complex provides a tool for delivery of host-targeted siRNA into anthrax pathogenesis-associated host cells, and it can be used as a lifesaving postexposure therapeutic vaccine against anthrax.

P.D4.10 Exploiting host pathogen interaction - Part 10

P.D4.10.01 Influence of regulatory T cells in immune answer of dogs with visceral leishmaniasis

P. H. L. Bertoš, P. R. Moreira, M. B. Conceição, R. O. Vasconcelos; Sao Paulo State University (UNESP), Jaboticabal, Sao Paulo, Brazil.

Introduction - The visceral leishmaniasis (VL) is a chronic zoonotic disease, marked by macrophages infection with Leishmania infantum, which is distributed to many organs. Parasitic load is very different in each organ, showing that some tissues are more susceptible to this parasite, as spleen. The regulatory T cells (Treg) can avoid pro-inflammatory answer, thus, evaluate your role in canine VL would be important. This way, the aim of this study was detect Treg cells in the popliteal and pre-scapular lymph node, liver, spleen and skin (nose and ear) of dogs naturally infected with L. infantum, in endemic area for VL. Methods - The dogs were distributed in two groups: infected (n=29) and control (n=5), this had dogs of free VL areas. Immunohistochemistry was used to detect Treg cells in the tissue (FoxP3 antibody, diluted in 1:2500). Results - The immunostaining of Treg cells was observed predominantly at cell nuclei. In lymph node it was seen more at cortical area, in liver at intralobular granuloma, in spleen, at white pulp and in skin, at granulomas present around the cutaneous appendages. Comparing these two groups by each organ, significant difference was observed for Treg cells load in nose (P=0.0118), spleen (P=0.0043) and pre-scapular lymph node (P=0.0214), much of these cells was seen in infected group. Conclusion - Lymphoid organs and skin maybe has some influence in Treg cells regarding parasite surviving.

Keywords: Parasite immunology, Regulatory cells, Veterinary immunology, Lymphoid organs.

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P.D4.10.02 Vitamin D deficiency as potential predisposition factor for sepsis development

M. Buc, M. Olejnovar, A. Dobisova, J. Koutoum, S. Blažíková, M. Bacová; 1Institute of Immunology, Bratislava, Slovakia, 21st Department of Anaesthesiology and Intensive Care Medicine, Comenius University Faculty of Medicine and University Hospital, Bratislava, Slovakia, 3Piešťany Laboratories, Ltd, Piešťany, Slovakia.

Introduction: Vitamin D hormonal actions influence mineral metabolism and skeletal health. However, vitamin D has an impact on function of the immune system, e.g. it acts as important stimulant for innate immune and enhances the antimicrobial effects of macrophages and monocytes. In the frame of our project to find biomarkers distinguishing sepsis from non-infectious SIRS, we paid attention to this hormone. Material and methods: We investigated 32 patients suffering from SIRS/sepsis. 5 ml of blood was taken into EDTA tubes on day of admission to the clinic and subsequently on days 2, 3, 5, 7 or exceptionally day 10. Different cytokines and inflammatory markers were investigated. Except them, plasma levels of 25-hydroxyvitamin D /25(OH)D/ were evaluated by electrochemiluminescent binding test and were correlated with levels of CRP and presepsin (sCD14) - all from the 1st sample. Results: We found significantly decreased levels of 25(OH)D in septic patients (N=25; 11.084±4.965 μg/l) compared to SIRS patients (N=7; 19.071±8.44 μg/l; P=0.0097). The significant lower levels of 25(OH)D were found also in the group of patients who did not survive (N=5; 6.540 ±3.966) compared to those, who survived the 7th day of hospital care (N=27; 13.996±1.416; P=0.0076). We also disclosed a correlation between the levels of 25(OH)D, CRP (P=0.0003), presepsin (P=0.0032), and SOFA (sequential organ failure assessment) score (p=0.0385). Conclusions: Our results indicate that low levels of vitamin D predispose patients to the development of sepsis and influence their survival.

P.D4.10.03 Depletion of regulatory T cells in ongoing Paracoccidioidomycosis reverses disease severity

N. Galdino, F. V. Loures, E. F. Araujo, T. A. Costa, V. L. G. Calich; Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

In many infectious diseases, the suppressive activity of Treg cells has been associated with deleterious effects, however in certain experimental settings this activity can be protective due to the control of excessive inflammation. In paracoccidioidomycosis, the most important deep mycosis in Latin America, most studies on Treg cells function were performed by depleting Treg cells before or early in the infection. However, human PCM is diagnosed late, when the disease is already established. This fact led us study the effect of Treg cells depletion in a model of ongoing pulmonary PCM. Then, Treg cells depletion was performed by treatment of C57BL6/JOTG/GFP (DEREG) mice with diphtheria toxin (DT) after 3 weeks of infection with 1x10^4 Paracoccidioides brasiliensis yeasts by the intratracheal route. At weeks 6 and 10 after infection, DT treated DEREG mice showed reduced number of Treg cells that was associated with decreased fungal burdens in the lungs, liver and spleen as well as diminished tissue pathology when compared with control mice (infected and treated with saline). Additionally, DT treated mice showed an increased influx of CD4+ and CD8+ effector cells into the lungs paralleling the increased production Th1 and Th17 cytokines and reduced mortality. Altogether, our data demonstrate for the first time the beneficial effects of Treg cells depletion in established PCM. This procedure ameliorated all parameters of disease severity and immunity. More importantly, these findings indicate that the control of Treg cells in the course of PCM can be explored as a novel immunotherapeutic procedure.

P.D4.10.04 Identification of Borrelia burgdorferi phagocytic receptors and their role in the inflammatory response


Borrelia burgdorferi, the causative agent of Lyme disease, causes a range of inflammatory complications including arthritis, carditis and immune system disorders that can become long lasting if not properly treated. In the context of carditis, as the heart presents little exposure to antibodies, the control of the infection relies majorly on the phagocytic activity of macrophages. In turn, phagocytosis is required for the full response of macrophages including the production of proinflammatory factors. In spite of its importance, little is known about the complement of receptors and signals that mediate the internalization of B. burgdorferi. Through the use of transcriptomic and proteomic approaches we identified potential phagocytic receptors implicated in the clearance of B. burgdorferi in human and mice, with the differential capacity to induce pro-or anti-inflammatory signals. As a result, we have found that Fc Receptor CD64 seem to play an important role in the clearance of the bacteria and in the inflammatory outcome. Moreover, we have described the regulatory role of the TLR family member, CD180, which influences Borrelia burgdorferi phagocytic receptor CR3, and is implicated in the complex pathogen-host defense equilibrium. The identification of the full complement of phagocytic receptors and their pro/anti-inflammatory activity will allow to define internalization alternatives and, in the future, devise novel strategies to increase phagocytosis without a consequent intensification of local inflammatory responses.
Low CCR5 expression protects specific CD4+ T cells of HIV controllers from viral entry


1Virus and Immunity Unit, Pasteur Institute, PARIS CEDEX 15, France, 2INSERM U1018, Paris, France, 3Bioinformatics and Biostatistics Hub, Pasteur Institute, PARIS CEDEX 15, France, 4INSEM U1018, Center for Research in Epidemiology and Population Health, Le Kremlin-Bicêtre, France, 5HIV Unit, Foch Hospital, Suresnes, France, 6AP-H, Infectious and Tropical Diseases Department, Raymond Poincaré Hospital, Garches, France, 7INSERM U1184, Center for Immunology of Viral Infections and Autoimmune Diseases, Le Kremlin-Bicêtre, France, 8AP-H, Department of Internal Medicine and Clinical Immunology, University Hospital Paris Sud, Le Kremlin-Bicêtre, France, 9Université Paris Sud, UMR1184, Le Kremlin-Bicêtre, France.

HIV controllers, who spontaneously contain HIV replication to very low levels, develop particularly efficient antiviral T responses. To gain insights into the contribution of the CD4 helper subset to HIV control, we characterized the differentiation status of HIV-specific CD4+ T cells at the single cell level. CD4+ T cells reactive with MHC-II tetramers specific for the most immunodominant HIV epitope (Gag293) were analyzed by multiplexed real-time qPCR combined with multiparametric flow cytometry. HIV controllers from the ANRS CODEX-CO21 cohort with a viral load <50 copies/ml were compared to efficiently treated patients with an equivalently low viral load. Gag293-specific cells from HIV controllers proved to express lower levels of PD-1 and of the HIV coreceptor CCR5 than those of treated patients, while CCL5 and TRBV2 expression were increased. Interestingly, HIV controller specific cells proved less susceptible to fusion with an HIV-JRF-L reporter virus (P=0.017). Moreover, CCR5 expression correlated with HIV fusion (R=0.83, P<0.005). CCR5 expression in total CD4+ T cells and the frequency of Gag293-specific cells, which comprised the subset of controllers with lower CCR5 expression maintained strong CD4 responses. Genetic analysis of one controller with particularly low fusion susceptibility uncovered biallelic mutations that impaired CCR5 expression. Taken together, these findings reveal a lower susceptibility of HIV controller specific CD4+ T cells to HIV entry, and point to a role for low CCR5 expression in promoting spontaneous HIV control.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

POSTER PRESENTATIONS

P.D4.10.10

Induction of neutrophil extracellular traps by Mycoplasma bovis and degradation of them by MnuA the major membrane exonuclease

F. Haile1, C. Hartley1, S. Sansom2, J. Coome3, P. Mansell4, D. Beggs5, G. Browning5

1Asia Pacific Centre for Animal Health, The University of Melbourne, Melbourne, Australia, 2Department of Veterinary Clinical Sciences, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Melbourne, Australia.

Mycoplasma bovis is recognised as an important pathogen of cattle and uses a range of cell surface proteins to evade the host immune system. We investigated the capacity of M. bovis to induce Neutrophil Extracellular Trap (NET) formation and the effect of the major membrane nuclease MnuA, which in vitro is responsible for the majority of the nucleosome activating function of M. bovis, on the NET formation. The wild type M. bovis PG64, the nuclease deficient mutant MBPG64_0215 (MnuA), the putative nuclease Δ310 deficient mutant, and the mnuA complemented strain (mnuA-PIR485) were grown in modified Frey’s broth and their nucleosome activities were compared using nuclease zymograms. Fluorescence microscopy was employed to visualise the presence of NETs in neutrophils isolated from healthy cows while a Sytox-Green based assay was used to quantify the formation of NETs. A luminal-based ROS assay was used to determine the role of reactive oxygen species (ROS) in the process of NET formation. NETs were detected following exposure of the wild type or the mnuA mutant to the wild type or the mnuA-recombinant complemented mutant, and NETs were decreased in the presence of even low concentrations of wild type M. bovis. Our study demonstrates that M. bovis can induce NET formation in bovine neutrophils, albeit in the absence of induction of ROS, but that the major membrane nuclease MnuA is able to rapidly degrade them, and thus is likely to play a significant role in virulence.

P.D4.10.11

Unraveling the regulation of T cell - pathogen equilibration during chronic infection

J. Handschuh1, M. Alabdullahi2, P. Formaglio3, L. Philippsen4, J. Mohr5, A. J. Müller5,6

1Institute for Molecular and Clinical Immunology, Magdeburg, Germany, 2Institut Pasteur, Paris, France, 3Heilmann Centre for Infection Research, Braunschweig, Germany.

Chronic infections require the escape of the pathogen from sterilizing immune responses, while the immune system may downregulate its effector functions to prevent damage at the cost of pathogen persistence. However, little is known about the mechanisms by which the immune response equilibrates with the pathogen in order to stabilize the infectious burden at a low level, which is key to permit chronic and often asymptomatic infection. Particularly, it is unknown how the interactions of effector and regulatory T cells (Teff and Treg) among each other, as well with the pathogen, impact the establishment of a persisting pathogen reservoir. We are using the intracellular parasite Leishmania major as a model for infections efficiently contained by T cells, yet persisting at the site of infection after resolution of pathology. The persisting reservoir is important for maintenance of immunity against reinfection and is dependent on the presence of Treg. We have set up in vivo biosensors in order to unravel the interplay of the T cell compartment and pathogen physiology at the site of infection by intravital 2-photon microscopy. This approach allows for probing not only pathogen viability, but also the activation of effector T cells during their dynamic interaction with Leishmania-infected phagocytes. By monitoring immune cell activation and pathogen clearance in the context of the cellular microenvironment at the site of infection, we now aim at elucidating how the equilibrium between pathogen containment and immune activation is realized during the persistence of Leishmania major.

P.D4.10.12

Investigating the capacity of Kupffer cells to acquire an innate memory function through sensing of apoptotic cells

I. Liebold1, L. I. Basurgo2

1University Hospital Eppendorf, Hamburg, Germany, 2Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Sickleocytes are trematodes that are responsible for Schistosomiasis. Around 240 million people are infected with the parasite worldwide. Upon host infection, the release of parasite eggs leads to granuloma formation in the liver and induces a type 2 immune response. Macrophages, which react to type 2 cytokines, such as IL-4 and IL-13, are responsible and necessary for the switch from a type 1 to a type 2 immune response. Although this switch is essential for the initial survival of the host, at later stages of the disease, macrophages are also associated to the induction of liver fibrosis. Interestingly, Kupffer cells (KCs), resident macrophages in the liver, are not only highly phagocytic cells but they also have a long-lived capacity. Here we hypothesize that, similar to long lived cells of the adaptive immune system, KCs can acquire an innate memory capacity, driven by the phagocytosis of apoptotic cells. We have recently described that sensing of apoptotic cells is essential for the response to type 2 cytokines and acquisition of a tissue remodeling function in macrophages. Here we show that in the liver, Kupffer cells express phagocytic receptors such as AXIN and MERTK, and can increase their tissue remodeling function upon sensing of apoptotic cells both in vivo and in vivo experimental settings. Understanding new mechanisms for regulating, KCs activation and induction of tissue remodeling may reveal novel therapeutic targets and provide crucial insights for long lasting protection against hepatic infections, while avoiding liver fibrosis. Founded by SFB841, Hamburg.

P.D4.10.13

Ovine C-type lectin receptor (CLR)-Fc fusion protein library - a novel tool in veterinary immunology to screen for virus/CLR interactions

D. Lindenwald1, K. Jung1, S. Becker2, S. Rautenschlein3, M. Buettner4, G. Alber4, B. Lepers5

1Immunology Unit & Research Center for Emerging Infections and Zoonoses (RIZ), University for Veterinary Medicine Hannover, Foundation, Hannover, Germany, 2Institute for Animal Breeding and Genetics & Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, Foundation, Hannover, Germany, 3Institute for Parasitology & Research Center for Emerging Infections and Zoonoses (RIZ), University for Veterinary Medicine Hannover, Foundation, Hannover, Germany, 4Clinic for Poultry, University for Veterinary Medicine Hannover, Foundation, Junckerstr., Hannover, Germany, 5Institute of Immunology/Molecular Pathogenesis, Center for Biotechnology and Biomedicine, College of Veterinary Medicine, University of Leipzig, Leipzig, Germany.

Innate immunity is the first line of defense against parasitic, bacterial, fungal and viral pathogens and depends on the recognition of evolutionarily conserved pathogen patterns by innate immune receptors. Among other pattern recognition receptors, C-type lectin receptors (CLRs) recognize pathogen-derived patterns and thus are important cross-linkers between pathogen containment and immune activation and therefore important for maintenance of immunity against reinfection and is dependent on the presence of Treg. In the liver, Kupffer cells are phagocytic cells that have a long-lived capacity. Here we hypothesize that, similar to long lived cells of the adaptive immune system, KCs can acquire an innate memory capacity, driven by the phagocytosis of apoptotic cells. We have recently described that sensing of apoptotic cells is essential for the response to type 2 cytokines and acquisition of a tissue remodeling function in macrophages. Here we show that in the liver, Kupffer cells express phagocytic receptors such as AXIN and MERTK, and can increase their tissue remodeling function upon sensing of apoptotic cells both in vivo and in vivo experimental settings. Understanding new mechanisms for regulating, KCs activation and induction of tissue remodeling may reveal novel therapeutic targets and provide crucial insights for long lasting protection against hepatic infections, while avoiding liver fibrosis. Founded by SFB841, Hamburg.

P.D4.10.14

Unraveling the regulation of T cell - pathogen equilibration during chronic infection

I. Liebold1, L. I. Basurgo2

1University Hospital Eppendorf, Hamburg, Germany, 2Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Sickleocytes are trematodes that are responsible for Schistosomiasis. Around 240 million people are infected with the parasite worldwide. Upon host infection, the release of parasite eggs leads to granuloma formation in the liver and induces a type 2 immune response. Macrophages, which react to type 2 cytokines, such as IL-4 and IL-13, are responsible and necessary for the switch from a type 1 to a type 2 immune response. Although this switch is essential for the initial survival of the host, at later stages of the disease, macrophages are also associated to the induction of liver fibrosis. Interestingly, Kupffer cells (KCs), resident macrophages in the liver, are not only highly phagocytic cells but they also have a long-lived capacity. Here we hypothesize that, similar to long lived cells of the adaptive immune system, KCs can acquire an innate memory capacity, driven by the phagocytosis of apoptotic cells. We have recently described that sensing of apoptotic cells is essential for the response to type 2 cytokines and acquisition of a tissue remodeling function in macrophages. Here we show that in the liver, Kupffer cells express phagocytic receptors such as AXIN and MERTK, and can increase their tissue remodeling function upon sensing of apoptotic cells both in vivo and in vivo experimental settings. Understanding new mechanisms for regulating, KCs activation and induction of tissue remodeling may reveal novel therapeutic targets and provide crucial insights for long lasting protection against hepatic infections, while avoiding liver fibrosis. Founded by SFB841, Hamburg.
P.D4.10.16
Analysis of the imbalance between regulatory B and T cells and circulating T follicular helper cells in HIV-infected patients

J. Lopez-Antéria,1 C. Güterres, V. Perez-Fernandez,1 A. Prieto-Sánchez,1 R. Correa-Rocha,1 S. Moreno-Guillen,1 M. Muñoz-Fernandez1, M. Pion1
1Instituto de Investigacion Sanitaria Gregorio Marañon, Madrid, Spain; 2Hospital Ramón y Cajal, Madrid, Spain; 3Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Background: HIV infection in vitro alters production rates of T (Reg) and B (Breg) subsets. These subsets play a crucial role in the maintenance of immune homeostasis, and it has been recently described that T follicular helper cells (Tfh) are pivotal for the development of Breg, and could also modulate the Treg maintenance.

Methods: We have analyzed the phenotypes of four different Breg subsets, and Treg and circulating Tfh (cTfh) compartments along with the frequencies of IL-10, IL-17, IL-4 and INF-y secreting cells in naïve-treated HIV+ patients, in treated-HIV+ patients and in healthy individuals. Finally, we analyzed the suppressive capacity of Breg from HIV-infected patients or healthy individuals.

Results: Absolute counts of Treg and Breg were decreased and frequency of cTfh was increased in naïve-treated HIV+ patients in comparison to treated-HIV+ patients or healthy individuals. Positive correlation between cTfh and Treg observed in healthy individuals were lost in naïve-treatment HIV+ patients, but surprisingly correlations between Breg subsets and Treng were established in naïve-treatment HIV+ patients in comparison to healthy individuals. Conclusions: We demonstrated that the balance between cTfh, Treg and some Breg subsets are deregulated in HIV-infected patients and that these cellular compartments might participate in the immune system hyperactivation and exhaustion. This work was supported by the Ministry of Economy and Competitiveness ISCIII-FIS grants PI12/01763, PI12/00934 and PI15/00923, co-financed by ERDF from the European Commission, “A way of making Europe”. A.P-S and V.P-F were supported by the Youth Employment Program co-financed by the Madrid community and FEDER Founds.

P.D4.10.17
An unusual presentation of hip pain in a patient with known hyper IgE syndrome and multiple calcified pelvic apophyses

A. Saad, S. Shahbon, T. ElGamal
Heart of England NHS trust, Birmingham, United Kingdom.

Introduction Hyper-IgE syndrome (HIES) is a relatively rare condition which, from childhood, renders patients susceptible to infection. Typically patients with HIES can develop various orthopaedic manifestations of this disease, namely, scoliosis, pathological fractures, osteoporosis and potentially septic arthritis. Case report We present the case of W, a 44-year-old patient with known HIES and a 7 week history of left hip pain. We discuss the clinical presentation, and the curvatures which came our way when investigating this patient and how we overcame them. We also demonstrate a very interesting pelvic radiograph from this patient which shows multiple sites of calcified apifications. Something which is firstly unexpected in such patients and secondly something not previously reported in the literature. Conclusion Several issues and conundrums can present themselves when dealing with patients known to have HIES. We demonstrate how we managed such a patient and maintained a high level of suspicion in such patient.

P.D4.10.18
RNA derived from Plasmodium falciparum and Litosomesoides sigmodontis is potent to induce a pro-inflammatory immune response in human cells

J. F. Scheunemann1, A. L. Neumann1, S. J. Frohberger1, A. Ehrens1, C. Coé1, M. P. Huebner1, A. Hoerauf2, B. Schumak2
1Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany, 2Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn, Germany.

Introduction The elimination of infectious diseases like malaria and filariasis is an important aim of the United Nations. Despite tremendous research progress regarding the understanding mechanisms of these parasitic diseases, it still remains elusive how initial parasite recognition works. In contrast to surface structures and parasite products, the role of their nucleic acids is weakly characterized. Here we investigate the potential of parasitic nucleic acids and corresponding host proteins as targets for successful disease modification.

Materials and Methods: We investigated the potential of P. falciparum and L. sigmodontis-derived RNA to evoke an immune response, compared to human and E. coli RNA, by in vitro cultures of human peripheral blood mononuclear cell (PBMC) and reporter cell lines. We quantified the secreted cytokines and analyzed cell activation by flow cytometry.

Results: ELISA revealed that cytosolic delivery of L. sigmodontis-RNA induced a strong induction of TNF-α in PBMCs, whereas P. falciparum-RNA was more stimulatory when delivered to the endosome, resulting in a cytokine pattern associated with NFκB activation. We further showed that monocytic cell populations were strongly activated after the challenge with parasitic RNA that was RIG-1 dependent.

Conclusion: Parasitic RNA derived from P. falciparum and L. sigmodontis harbors a pro-inflammatory potential, manifesting in the activation of monocytes, type I IFN secretion and presumably NFκB activation. The evoked immune response differs between endosomal and cytosolic recognition. The cytosolic RNA receptor RIG-I was crucial for recognition of parasitic RNA and subsequent cell activation.

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P.D4.10.19
Reverse immunology as a tool to identify broadly recognized pneumococcal proteins targeted by human T-cells

M. D. B. van de Garde, E. van Westen, M. C. Poelen, N. Y. Rots, C. A. van Els
Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIV, Bilthoven, Netherlands.

Introduction: T-cell mechanisms, which are implied in protection against pneumococcal colonization, should be unraveled to understand the mode of action of future universal protein-based pneumococcal vaccines. Here we apply reverse immunology to predict and verify broadly recognized human T-cells.

Methods: Hundred pneumococcal proteins of diverging subcellular localization were selected for in silico prediction of T-cell immunogenicity based on HLA-DR binding and absence of cross-reactivity against human proteins (Epivax). For 20 potentially T-cell immunogenic proteins, peptides predicted to bind +4 of 8 common HLA-DRB1 alleles were synthesized, pooled per protein and tested in T-cell proliferation and cytokine assays using PBMCs from a panel of healthy adults and (ex-) pneumococcal pneumonia cases.

Results: Peptide pools of 19/20 proteins evoked T-cell responses in healthy adults. Most frequent responses (in ≥ 25% of 20 donors tested) were found for SP_0117 (PspA), SP_0468 (putative sortase), SP_0546 (BlpZ), SP_1650 (PsaA), SP_1923 (pneumolysin), SP_2216 (PcsB), and SPR_0907 (PhtD). Healthy adults and cases had diverging patterns of protein immunodominance and cytokine profiles (IFNg, TNFa, IL-13 and IL17A). We demonstrated proof of principle for a reverse immunology approach to screen human pneumococcus specific T-cell responses at a semi-large proteome scale. Single peptides can evoke measurable proliferative and cytokine responses, including IL17A, thought to play a role in the protection against S. pneumoniae. Currently, in depth T-cell analyses are ongoing in pneumococcal carriers and (ex-) cases of various age groups.

P.D4.11.11
Probing host pathogen interaction - Part 11

P.D4.11.02
HIV-1 modulates TRAF proteins to promote pro-inflammatory condition and avert Interferon induction

S. Trivedi, A. C. Bonerjee
National Institute of Immunology, New Delhi, India.

The most obvious route for infection available to HIV-1 in humans is via epidermis, dermis and lymph nodes and doing so it encounters a lot of resistance as every nucleated cell it comes across can potentially mount an innate immune response against it. Especially, the tissue dendritic cells and macrophages where the virus faces various pattern recognition receptors (PRRs) before it can establish a productive infection. An important family of signaling adaptor proteins involved in different PRR pathways is TRAF (TNF-a Receptor Associated Factor) Family of proteins. Almost all PRRs involved in detection of HIV-1 converge down to TRAF family of proteins which in turn leads to differential effector functions.

On one hand, TRAF6 activates NF-κB and AP-1, which binds to the LTR region of HIV-1 and promotes its transcription, while on the other hand, TRAF3 activates IRF-3 and IRF-7 which promote synthesis of interferons and in turn provide anti-viral activity. Thus modulation of these two effector arms at the right time can be very beneficial for the virus and delineating this phenomenon can provide better insights into the pathogenicity of the virus. In our work we are able to show that HIV-1 modulates these key signaling adaptors - TRAF3 and TRAF6 to enhance its replication capacity in the cell. HIV-1 Vpr and Vpu proteins are involved in this modulation which down-regulate TRAF3 and up-regulate TRAF6 to a similar extent, thereby creating a pro-inflammatory environment which enables the virus to have a much more productive replication.
Suppressor of cytokine signaling-3 and control of progression toward liver fibrosis and hepatocellular carcinoma in chronic HCV-infected patients


1Institut Pasteur du Maroc, Casablanca, Morocco; 2Université Hassan II de Casablanca, Casablanca, Morocco; 3CHU Ibn Rochd de Casablanca, Casablanca, Morocco; 4Institut Pasteur de Paris, Paris, France.

Chronic Hepatitis C is one of the most important risk factors of liver cirrhosis and hepatocellular carcinoma. Before reaching these ultimate steps, insulin resistance triggered by hepatitis C virus infection is known to participate in the progression of liver disease. The present study aims to investigate the influence of two functional polymorphisms on SOCS3 mediated restriction in dysregulated hepatic fibrosis and on the outcomes of CHC progression in a North African context. In this case-control study, 601 Moroccan subjects composed of 300 healthy controls, 101 resolvers and 300 patients with persistent HCV infection including 95 mild chronic hepatitis, 131 Advanced Liver Diseases and 74 HCC were enrolled. They were genotyped for the 4874 A/G (rs4967170) and A930>T (rs4961618) SOCS3 variants using TaqMan SNPs assays. SOCS3 mRNA expression was assessed using Real Time PCR technique. Logistic regression analysis showed that variation at rs4969168 was associated with spontaneous clearance of HCV (P<0.05). In addition, minor allele frequencies were significantly higher in AD-LD patients when compared to the matched group both for rs4961618 (P=7.7 E -04) and rs4966170 (P=4.9 E -05). A significant association between haplotype and liver disease progression was also found. Moreover, SOCS3 mRNA was significantly more expressed in peripheral leukocytes from patients with HCC than in those from mCHC. Finally, rs4969170 was significantly associated with LDL- lip protein (P=0.04), total cholesterol (P=5.0 E -04) and the highest fasting glucose levels (P=0.005) in patients with persistent HCV infection. Our results underline the importance of the functional SOCS3 polymorphisms in the modulation of HCV progression and suggest their contribution to HCC development by affecting its mRNA expression and perturbing key metabolic parameters.

Phagocytosis of non-motile Pseudomonas aeruginosa

S. Demirdjian, D. Hopkins, H. Sanchez, B. Berlin;
Dartmouth College, New Hampshire, United States.

Pathogenic bacteria that establish chronic infections in immunocompromised patients frequently undergo adaptation or selection for traits that are advantageous for their growth and survival. Clinical isolates of Pseudomonas aeruginosa, a gram-negative, opportunistic bacterial pathogen, exhibit a temporal transition from a motile to a non-motile phenotype through loss of flagellar motility during the course of chronic infection. This progressive loss of motility is associated with increased resistance to both antibiotic and immunological clearance. We have previously shown that loss of bacterial motility enables P. aeruginosa to evade phagocytic clearance both in vitro and in vivo and fails to activate the Phosphatidylinositol-3-Kinase (PI3K)/Akt-dependent phagocytic pathway. Therefore, we tested the hypothesis that clearance of phagocytes-resistant bacteria could be induced by exogenously pre-treating innate immune cells with the Akt activating molecule phosphatidylinositol-(3,4,5)-trisphosphate (PIP3). Here we demonstrate that PIP3 induces the uptake of non-motile P. aeruginosa by primary human neutrophils 25-fold, and this effect is phenocopied with the use of murine phagocytes. However, surprisingly, mechanistic studies revealed that the induction of phagocytosis by PIP3 occurs because polyphosphoinositides promote bacterial binding by the phagocytes rather than bypassing the requirement for PI3K. Moreover, this induction was selective, since the uptake of other non-motile gram-negative, but not gram-positive bacteria, can also be induced by PIP3. Since there is currently no treatment that effectively eradicates chronic P. aeruginosa infections, these findings provide novel insights into a potential methodology by which to induce clearance of and bacteria and to abrogate the inflammatory response to phagocytose recognition of P. aeruginosa. NIH (P30 RO123126-01, R21 AI121820), Cystic Fibrosis Foundation (STANT01980, STANT01180).

Estrogen stimulates phagocytosis by macrophages in both in vitro and ex vivo models of age-related impaired healing via the estrogen-receptor alpha

M. El Mohtadi, K. Whitehead, A. Fadel, J. Ashworth;
School of Healthcare Science, Manchester, United Kingdom.

Annual expenditure for the treatment of chronic wounds in the elderly exceeds $9 billion. Chronic wounds are frequently colonised by opportunistic pathogens such as Staphylococcus aureus and Pseudomonas aeruginosa, and declining levels of estrogen with increasing age delays healing. This study investigated the effect of hormonal aging (estrogen deprivation) on the clearance of methicillin-resistant S. aureus (MRSA) and P. aeruginosa by macrophages derived from U937 and human primary CD14+ monocytes. Concentrations of 17b-estradiol were used to model estrogen levels found in the elderly (estrogen deprivation: absolute absence and 1x10-3M), young adults (1x10-9M), and following exogenous supplementation (1x10-7M). The estrogen receptor (ER) isoform(s) involved in bacterial clearance were determined using selective ER modulators. Estrogen concentrations at typical levels of young or supraphysiological levels significantly (P<0.05; n=24) increased the phagocytosis of MRSA and P. aeruginosa in a concentration-dependent manner compared to estrogen deprivation. Confocal and scanning electron microscopy confirmed estrogen increases co-localisation of fluorescent GFP-S. aureus or mCherry-P. aeruginosa with macrophages and promotes bacterial internalisation. ER-alpha (ERa) activation mirrored the stimulatory effect of estrogen on phagocytosis whilst ERa antagonism completely blocked the effect of estrogen. In contrast, activation or antagonism of ER-beta (ERb) had no effect on phagocytosis, confirming estrogen mediates bacterial clearance via ER-alpha.

These findings suggest estrogen promotes the resolution of wound bacteria during youth but this protection is lost as estrogen levels decline with increasing age. Novel dressings that provide estrogen supplementation or selective activation of ER-a may be an effective treatment option for colonised wounds in the elderly.

Chlamydial co-infection boosts the CTL-stimulatory capacity of HCV-1-exposed DCs in a time-dependent manner

M. Schönfeld1, U. Knackmuss1, P. Chandorkar3, T. J. Hope4, A. Marris1, R. Bellmann-Weiss5, C. Less-Fröh1, W. Dilljöng7, W. Posch3

1Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Innsbruck, Austria; 2Central Institute for Blood Transfusion & Immunological Department, Innsbruck, Austria; 3Institute for Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, United States; 4Centre d’Immunologie et des Maladies Infectieuses-Paris, Pierre et Marie Curie University (UMRS C7), INSERM U1135, CNRS U825, Paris, France; 5University Hospital for Internal Medicine II, Medical University of Innsbruck, Innsbruck, Austria.

Pathogenic bacteria and their microbial products activate dendritic cells (DCs) at mucosal surfaces during sexually transmitted infections (STIs) and might also modulate their functions during infections with HIV-1. We recently illustrated that complement (C) coating of HIV-1 (HIV-C) as found during the acute phase of infection by-passed SAMHD1-mediated restriction in DCs and thereby mediated an increased DC activation and antiviral capacity. To determine whether the superior antiviral effects of HIV-C-exposed DCs also apply during bacterial co-infection, we developed a super-infection model in which DCs were infected with Chlamydia spp. simultaneously (HIV+Chlam-DCs) or followed by HIV-1 infection (Chlam-DCs). Simultaneous infection of DCs with HIV-1 and Chlamydia significantly boosted the CTL-stimulatory capacity compared to HIV-C-loaded DCs. This protective effect was lost upon pre-infection with Chlamydia 3h or 24h prior addition of opsonized HIV-1. The reduction in the CTL-stimulatory capacity was not due to lower HIV-1 binding, internalization or infection of Chlam-DCs compared to DCs HIV+Chlam-DCs, but due to altered fusion and internalization mechanisms within DCs. CTL-stimulatory capacity of HIV-C in Chlam-DCs correlated with significantly reduced viral fusion than DCs HIV+Chlam-DCs and illustrated considerably increased numbers of HIV-containing vacuoles compared to DCs. These data indicate that Chlamydia super-infection of DCs mediates a transient boost of their HIV-specific CTL-stimulatory and antiviral capacity, which is reversed in a time-dependent manner.

Hyperinduction of Interferon Lambda and proinflammatory cytokines upon injection of neural cells with Zika virus

A. S. M. SEJUM, S. M. Lee

The University of Hong Kong, Hong Kong, Hong Kong.

In Feb, 2016, The WHO has declared Zika virus as a “Public Health emergency of international concern” after the outbreak in Brazil were numerous cases of microcephaly in newborn of Zika infected mothers as well as acute myelitis in adults. Zika is an enveloped, non-segmented, positive sense, single stranded RNA virus. Here we investigated the viral replication kinetics and the host immune response in human neural cells after Zika virus infection. Human differentiated astrocytes (d T89G), neuronal (d SH-SY5Y) and microglial (immortalized Human Microglia-SV40) cells were infected with Zika virus at MOI of 4. TCID50 was used to measure the viral replication kinetics. We found that Zika replicated efficiently in the neuronal and microglial cells but to a lesser extent in astrocytes. RT-qPCR was used to measure the host immune gene responses in astrocytes. We found that Zika induced type 1 interferon represented in IFN-beta induction in microglial and astrocytic cells. Interestingly, we found that Zika induced the expression of type III interferons, IL-29 in astrocytic and microglial cells and IL-28b in neuronal and microglial cells as well as IFN lambda receptor (IL10R9). Proinflammatory cytokines and chemokines hyper induce in Dharmacon with RAVNTE, PI10, IL8, IL6 and CCL2. Hyperinductions of pattern recognition receptors involved in viral recognition were identified with TLR3 and RIG-I. Our results demonstrated that three of the main neural cell types are susceptible to Zika virus infection. The hyper-induction of IL-29 in astrocytic and microglial cells opens new insights for Zika host antiviral response.
POSTER PRESENTATIONS

P.D.4.11.08
Blood transcriptomic profiles to differentiate enteroviral meningitis from bacterial meningitis
E. Bartholomeusz1, N. De Neuter1,2, A. Lemay3, D. Tuerlinckx4, W. Van Hove1, E. Van Den Steen1, T. Jonckheer1,2,6, V. De Pauw1, K. Van der Molen1,2, A. Lemay3, C. De Geest1, N. Loyts4, K. Van der Molen4, C. J. Kenyon5, M. Verma6, K. E. Chapman6, F. Schilt7, K. De Bosscher7, G. Opdenakker6, P. E. Van den Steen1
1The Peter Doherty Institute for infection and immunity, Melbourne, Australia, 2Institute of Experimental Immunology, Bonn, Germany.

CD1 T cell priming relies on the ability of dendritic cells (DC) to present antigen as well as recognizing, processing and communicating contextual cues associated with antigen presentation to CD1 T cells. DC often require CD40-CD40L-mediated interactions with helper CD4 T cells to generate an efficient CD1 T cell response. Precisely how such a “T cell help” is provided, and how it optimizes the priming capacity of DC remains uncertain. Precisely how the CD1 T cell help ameliorates the capacity of DCs to generate efficient priming of CD1 T cell in response to suboptimal antigenic stimuli must be determined. Focusing on the CD40-driven activation of innate pathways induced in IFN-α/β-stimulated DC, this study was designed to decipher the molecular mechanisms underlying such help phenotype. We have observed different patterns and dynamics of a fast amplification of various cytokines such as IL-15, IL-6 and IL-12p40, amplification induced by CD40 treatment following IFN-α stimulation. Moreover, transcriptomics, proteomics and protein phosphoproteomics analysis suggest that this cross-talk between IFN-β and T cell signal was integrated through the distinct stimulation of particular aspects of the NF-κB pathways. These findings argue for a complex synergism between CD40 signaling and innate stimuli that enable DCs to flexibly adjust cytokine secretion to the strength of inflammatory responses that accompany the acquisition of antigen. By dissecting how T cell help amplifies innate signals required for CD1 T cell priming, this work will assist in the development of more targeted T-cell-based therapeutic strategies.

P.D.4.11.10
Adrenal hormones mediate disease tolerance in malaria
L. Vandermosten1, T. Pham2, S. Knoops3, C. De Geest1, N. Loyts4, K. Van der Molen4, C. J. Kenyon5, M. Verma6, K. E. Chapman6, F. Schilt7, K. De Bosscher7, G. Opdenakker6, P. E. Van den Steen1
1Rega Institute for Medical Research, KU Leuven - University of Leuven, Leuven, Belgium, 2The Queen’s Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom, 3KU Leuven - University of Leuven, Leuven, Belgium, 4VIB Center for Medical Biotechnology, Ghent University, Ghent, Belgium.

Malaria, a global parasitic disease with severe complications, reduces host fitness and survival by pathogen-mediated damage and/or exaggerated host inflammation. Disease tolerance mechanisms counter these negative effects without interfering with pathogen load and thereby improve host survival. Adrenal hormones, including glucocorticoids (GCs) and adrenalins, have several homeostatic functions. GC levels are increased in patients with malaria, but their precise role remains unknown. In four different mouse models of malaria, we demonstrated that adrenal hormones protect mice against early death during infection, independent of parasitemia and parasite-mouse strain combinations. Adrenal hormones thus confer disease tolerance, leading to less severe symptoms. Surprisingly, adrenalins differentially affected malaria-induced inflammation by increasing circulating cytokine levels and inducing tissue pathology, but not in the brain, but not in the liver or lungs. Furthermore, without effects on the hepatic glucocorticoid enzyme transcription and the free fatty acid levels in plasma, adrenalins caused lethal hypoglycemia upon infection, independently from TGF-α and insulin. Glucose administration did not prevent or reverse lethal hypoglycemia. In contrast, treatment with a synthetic GC (dexamethasone) prevented the hypoglycemia, lowered cerebral cytokine expression and significantly increased the survival rates. Overall, we conclude that adrenal hormones do not protect against lung and liver inflammation in malaria, but instead protect against systemic and brain inflammation and severe hypoglycemia. Funding: Research Foundation Flanders (FWO-Vlaanderen) and the Research Fund (Geconcerteerde Onderzoeksacties G0A 2013/014 and C1 project 16/17/010) of KU Leuven. TP holds an aspirm PhD fellowship of the F.W.O.-Vlaanderen and PVdS is a Research Professor at the KU Leuven.

P.D.4.11.11
TOB1 inhibits IRF3-directed antiviral responses by recruiting HDAC8 to specifically suppress IFN-β expression
Z. Yu, M. Jo, W. Zhao;
Department of Immunology, School of Basic Medical Science, Shandong University, China, Jinan, China.

Innate immunity is the first line of host to defense against viral invasion and need to be precisely controlled. Viral infection induced type I interferons (IFNs) production play fundamental roles in innate immunity against virus and maintain immune homeostasis. However, the epigenetic regulatory mechanisms of type I IFNs production is unclear. The transducer of ErbB-2.1 (TOB1) is a member of the anti-proliferative family of BIG/TOB (B cell migration factor/erbB2). TOB1 plays crucial regulatory roles in T cell activation and cancer, via interacting with SMAD4 or SMAD2. However, its potential roles in innate immunity is unknown. In the present study, we found that the crystal structures of SMAD and IRF3 are very similar, and TOB1 could interact with IRF3. TOB1 expression could be markedly induced during viral infection in macrophages. TOB1 deficiency enhances both RNA and DNA viral-induced IFN-β production, and inhibits viral replication in macrophages. Mechanistically, TOB1 associates with IRF3, recruits HDAC8, and promotes HDAC8 binding to IFN-β promoter. Thus, TOB1 attenuates the acetylation of histone in the IFN-β promoter region, and thus inhibiting IFN-β transcription. Therefore, we identified TOB1 as a negative regulator of IFN-β production and outlined a new feedback mechanism for the epigenetic control of antiviral immune responses.

P.D.4.11.12
Sclafen 14 (SLFN14) is a novel antiviral factor involved in the control of viral replication
G. Shin;
Korea University School of Medicine, Seoul, Korea, Republic of.

Sclafen (SLFN) proteins have been suggested to play important functions in cell proliferation and immune cell development. In this study, we determined the antiviral activities of putative RNA helicase domain-containing SLFN14. Murine SLFN14 expression was specifically induced by TLR3-mediated pathways and type I interferon (IFN) in RAW264.7 mouse macrophages. To examine the role of SLFN14 during viral infection, cells were infected with either wild-type PR8 or dSN1/PR8 virus. SLFN14 expression was specifically induced following influenza virus infection. Overexpression of SLFN14 in A549 cells reduced viral replication, whereas knockdown of SLFN14 in RAW264.7 cells enhanced viral titers. Furthermore, SLFN14 promoted the delay in viral NP and M expression from cytoplasm to nucleus and enhanced RIG-I-mediated IFN-β signaling. In addition, SLFN14 overexpression antagonized the ability of HIV-1 to use host DNA virus (ZDV). In conclusion, our data suggest that SLFN14 is a novel antiviral factor for both DNA and RNA viruses.
When compared to the controls, some pro-inflammatory cytokines were unaltered (IL-1β, IL-12(p70), IL-23) due to infection, whereas levels of CCL5 and TGF-β1 were significantly increased. Samples were collected two times during the disease course. The mean levels of 16 cytokines and chemokines were determined by multiplex immunoassay with magnetic beads. They are also considered as one of the target cells for hantaviruses. The aim of our study was to analyze kinetics of the monocytes/macrophages related cytokines/chemokines in disease is an important. Hemorrhagic fever with renal syndrome (HFRS) is a viral disease caused by hantaviruses, however, it is primarily considered as an immune-mediated disease.

Changes in circulating cytokine and chemokine levels have been associated with many human diseases, and thus understanding the relationships between these changes and disease is important. Hemorrhagic fever with renal syndrome (HFRS) is a viral disease caused by hantaviruses, however, it is primarily considered as an immune-mediated disease.

Hantaviruses are single stranded, negative-sense RNA virus and are known to cause hemorrhagic fever with renal syndrome. The aim of our study was to analyze kinetics of the monocytes/macrophages related cytokines/chemokines in patients with hemorrhagic fever with renal syndrome.

They are also considered as one of the target cells for hantaviruses. The aim of our study was to analyze kinetics of the monocytes/macrophages related cytokines/chemokines in patients with hemorrhagic fever with renal syndrome.

The data indicate that under immunosuppressed conditions, rBCGmIL-18 displayed better ability than BCG to increase the percentage of T cells in mice immunized with mycobacteria for 6 and 12 weeks. Conclusion: Our results indicate that under immunosuppressed conditions, rBCGmIL-18 displayed better ability than BCG to increase the percentage of T cells in mice immunized with mycobacteria for 6 and 12 weeks.

The cytokine/chemokine profile indicates that under immunosuppressed conditions, rBCGmIL-18 displayed better ability than BCG to increase the percentage of T cells what may extend the knowledge in understanding the protective response of memory T cells in immunosuppressed conditions.

Our results indicate that under immunosuppressed conditions, rBCGmIL-18 displayed better ability than BCG to increase the percentage of T cells in mice immunized with mycobacteria for 6 and 12 weeks. Conclusion: Our results indicate that under immunosuppressed conditions, rBCGmIL-18 displayed better ability than BCG to increase the percentage of T cells in mice immunized with mycobacteria for 6 and 12 weeks.
Dengue virus infects epidermal human Langerhans cells for transmission to dermal dendritic cells

L. C. Helgers, J. K. Sprieholt, T. B. Geijtenbeek;
AMC, Amsterdam, Netherlands.

Dengue virus (DENV) is an enveloped positive ssRNA flavivirus that infects 390 million people on an annual basis. During recent years, studies have focused on DENV infection of dendritic cells (DCs), macrophages and monocytes. However, DENV is introduced into the human epidermis via mosquitoes bites. Therefore, a specialized subset of epidermal DCs called Langerhans cells (LCs) might be a target for DENV. Hence, this study investigates the role of LCs in DENV infection using ex vivo skin model. Isolated migratory LCs (migLCs) or sheets of epidermal human skin (ex-vivo LCs) were exposed to DENV-2 for 48h to determine infection. To research DENV-2 transmission, DENV-2 infected migLCs or ex-vivo LCs were added to DCs for another 48h. Exposure of epidermal skin to DENV-2 ex vivo led to infection of LCs. Furthermore, isolated migLCs were also efficiently infected by DENV-2. Strikingly, both migLCs and ex-vivo LCs transmitted DENV-2 to DCs, leading to high infection of DCs. Interestingly, transmission was dependent on both active DENV-2 infection of LCs and cellular interaction between LCs and DCs. These results demonstrate that LCs are not only permissive for DENV-2 infection, but also play a role in the transmission of DENV-2 to DCs. Normally, LCs fulfill a protective role against invading viruses by degrading viruses such as HIV-1. In contrast, this study provides evidence for a LC-dependent transmission route for DENV-2 infection of DCs and potentially other professional phagocytes. Therefore, preventing infection of LCs might limit dissemination of the virus throughout the host.

P.E1.01 Visualizing immune responses - Part 1

P.E1.01.01 Molecular imaging of retargeted UniCAR T cell therapy

N. Bernad1, S. Alberti1, C. Amador2, S. Karsita3, A. Feldmann1, M. Bachmann1,2, R. K. Bergmann1,2;
1Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany, 2University Medical Center Freiburg, Department of Immunology, Freiburg, Germany, 3University Medical Center Freiburg, Department of Virology, Freiburg, Germany.

The switchable UniCAR platform avoiding “off target” side effects by consisting of two components: UniCAR-modified T cells and specific targeting modules (TMs). For personalized precise imaging, molecular imaging approaches in vivo localization and kinetics of these components is mandatory. In this presentation, we demonstrate the optical (OI), MRI and PET imaging of this therapeutic concept in a preclinical setting using EGFR positive tumors in mice. UniCAR 281T cells were labeled with fluorescent nanoparticles and the cell line A431 was transwerected with the firefly luciferase. For PET imaging studies both, mono- and bivalent α-EGFR TMs were conjugated with [18F]FDG and radioabeled with [131I]. The bivalent α-EGFR TM showed an improved redirection of UniCAR T cells against EGFR carcinoma cells and was able to bind cell lines expressing high to low levels of EGFR. The killing of the tumor cells was visualized and quantified by luminescence imaging. In biodistribution studies both, mono- and bivalent α-EGFR TMs showed specific tumor accumulation that was time-dependent and to muscle ratios of more than 12, however, at the next day times and tumor to blood ratios of the bivalent α-EGFR was higher. The T cells were transiently accumulated in the lungs before they were distributed and also accumulated in lymph nodes. The study shows, that preclinical molecular imaging of all components of the retargeted UniCAR T cell therapy is possible and has the potential to be translated into the clinic.

P.E1.01.02 Neutrophil migration via lymphatic vessels in Aspergillus fumigatus-induced inflammation

M. Shevchenko, A. Fedorina, B. Boldkhovitina, A. Bogorodsky, V. Barshcheyevy, A. Sapozhnikov;
1Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation, 2Moscow Institute of Physics and Technology, Dolgoprudny, Moscow region, Russian Federation.

Neutrophils migrate fast from bone marrow to the site of inflammation. Recent studies have demonstrated that neutrophils can also relocate from the site of inflammation to lymph nodes via lymphatic vessels. Here we investigate the process of neutrophil migration during A. fumigatus infection.

For the research we used three-dimensional visualization of whole-mount conducting airways of mice at different time points after A. fumigatus inhalation. Tissue samples were stained against Mac3, Ly6G, Lyve-1 and CD31. Simultaneous visualization of neutrophils, lymphatic vessels and blood vessels was achieved by spectral unmixing. The optimal sets of primary and secondary antibodies were chosen and simultaneous staining of neutrophils with rat anti-Ly6G, blood vessels with goat anti-CD31 and lymphatic vessels with rabbit anti-Lyve-1 in combination with secondary donkey anti-rabbit-Alexa555 and donkey anti-rabbit-Alexa647 was performed. We visualized neutrophils that were associated with blood vessels in 6 hours after A. fumigatus conidia application. Examination of mouse bronchi in 72 hours after conidia inhalation revealed neutrophil association with lymphatic vessels. Thus, neutrophils were detected in lymphatic vessels at the late stage of A. fumigatus conidia application-induced immune response. The observation confirms the ability of neutrophils to migrate from the site of inflammation via lymphatic vessels.

The study was supported by RFBR № 18-315-00166

P.E1.01.03 Time course of different apoptotic stages during target cell killing

Biophysics, CIPMM, Homburg, Germany.

Introduction: CD8+ T- and NK cells are key players for elimination of cancer cells. Cancer target cells can be induced by two pathways: either perforin-dependent via lytic granules or receptor mediated via Fas-FasL interaction. Both pathways can lead to apoptosis, whereas only perforin can induce direct target cell necrosis (lysis). The apoptotic pathway involves the activation of caspases leading to cytoskeletal breakdown, DNA fragmentation and loss of membrane integrity. Nevertheless the kinetic progression of the different apoptotic stages during target cell killing by immune effector cells is not well understood.

Methods: We investigated apoptosis induction by primary NK cells and melanoma-specific CD8+ T cells clones in K562 and Jurkat T cells or T2 and melanoma cells respectively. We analyzed caspase activation and membrane disruption using amongst others the FRET-based cell death sensor Casper3-GR and the phosphatidylinerine sensor AnnexinV.

Experiments were performed using flow cytometry and fluorescent live cell imaging.

Results: We describe different apoptotic stages induced by either chemical substances or lymphocyte-mediated killing. As expected, detection of caspase activation is an early apoptotic event. AnnexinV, commonly used as apoptosis marker, on the other hand provides signals which appear in rather late stages of targeted cell death.

Conclusion: The detection of caspase activity during target cell killing to analyze apoptosis is an earlier marker compared to AnnexinV. Moreover the cell death sensor Casper3-GR enables a specific analysis of cytotoxic mechanisms by providing information about target cell morphotype and a precise discrimination of apoptotic and necrotic killing on single cell level.

P.E1.01.04 Cryoglobulin clearance after direct acting antiviral -DAA- therapy in hepatitis C virus HCV-monoinfected and HIV-HCV coinfected patients

M. N. Kolopa Sarda1, K. Hartig-Lavie3, P. Mialheva4, A. Uhrès5, V. Virlogieux3, P. Pradat3, F. Zoulim3, A. Bogorodskiy1, M. Bachmann1,2, R. K. Bergmann1,2, A. Sapozhnikov1;
1Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation, 2Moscow Institute of Physics and Technology, Dolgoprudny, Moscow region, Russian Federation, 3Chapelle Hospital, Lyon, France, 4University Claude Bernard Lyon, Faculty of Medicine, Lyon, France, 5University of Geneva, Faculty of Medicine, Geneva, Switzerland.

Background: Mixed cryoglobulins (MC) are found in 40-60% of patients with chronic HCV infection. The objective of this study was to evaluate the efficacy of DAA therapy on cryoglobulin clearance in patients with HCV-associated MC. Methods: HCV mono-infected and HIV-HCV co-infected patients with symptomatic or nonsymptomatic MC, who received DAA treatment from 2013 to 2016. Cryoglobulin was quantified by radial immunodiffusion. Results: 41 patients were analyzed: 31 HCV mono-infected patients and 10 HIV-HCV co-infected patients. MC was symptomatic in 77.4% of HCV mono-infected patients and in 10% of HIV-HCV co-infected patients. Two patients had Waldenstrom macroglobulinemia (WM) and 2 rheumatoid arthritis (RA). The most frequent symptoms were arthralgia, asthenia and polyneuropathy. DAA therapy was persistent in patients with WM and RA. Baseline cryoglobulin level was not associated with a protective effect against viral clearance during DAA treatment. Similarly, the presence of symptomatic MC, fibrosis stage and HCV genotype were not associated with cryoglobulin clearance.

Conclusions: DAA therapy allows a high rate of cryoglobulin clearance both in HCV monoinfected and HIV-HCV coinfected patients.
Natural killer (NK) cells are specialized lymphocytes with innate ability to eliminate virally infected and cancerous cells, but the mechanisms that control NK cell development and cytotoxicity are incompletely understood. We have identified novel roles for schistosomain domain-containing-I (Sostdc1) in NK cell development and function. Sostdc1 knock-out (KO) mice display a progressive accumulation of transitional NK cells (CD27+CD11b+, TNK) with age, indicating a partial developmental block. We simulated developmental rates with a deterministic compartmental model under the assumption that proliferation and death rates remain constant. Our model elucidates a requirement for Sostdc1 in NK cell development. Furthermore, we identified that Sostdc1-KD splenic Tnks express lower frequencies (%) of inhibitory Ly49G2, but higher % of activating Ly49H1+ and D+ cells. However, the % of Ly49H, +62, +H and +D populations were universally decreased at the mature (CD27- CD11b+, mNK) stage. We hypothesized that the Ly49 repertoire and developmental block in Sostdc1-KO mice would correlate with NK killing ability. We observed that KO NK cells are hyporesponsive against MHC-I-deficient cell targets in vitro and in vivo, despite having similar surface marker levels and similar IFN-γ gene expression. We plan to use high-dimensional flow cytometry to correlate IFN-γ gene expression to IFN-γ protein expression in NK cells cultured from Sostdc1−/− and wild type mice. Taken together, these data support a role for Sostdc1 in the regulation of NK cell development and could provide insights into novel biological parameters to increase active NK cell numbers with high killing efficiency for immunotherapies.
POSTER PRESENTATIONS

P.E1.01.11

Full automated load-and-go flow cytometry in neutrophil analysis: the start of a new era

Introduction: Changes in functional phenotypes of neutrophils is an adequate measurement of the amplitude of the innate immune response. A combination of these changes can be applied to predict infectious complications. Due to technical and logistical difficulties this concept is currently not clinically applicable. A fully automated 24/7 load and go flow cytometer would provide the opportunity to design such an analysis. Therefore, the aim of this study was to investigate the applicability of 24/7 automated analysis of neutrophils by flow cytometry. Methods: For proof of principle blood was drawn from healthy controls next to the flow-cytometer. Neutrophil activation was measured by the use of the automated AQUIOS load-and-go flowcytometer. The AQUIOS is able to pierce the tube caps, add antibodies, lyse and measure the sample within 20 minutes immediately after vena puncture. Thereafter, the same blood tubes were measured every 15 minutes. Measurements were done in presence or absence of the bacterial stimulus fMLF. Results: A significant increase in MFI was detected in the activation markers CD11b(174%146%-213% P=0.004), CD11b(245%(167%-524%), P=0.009) and CD11c(220%(153%-389%), P=0.008) within the first hour after vena puncture. After 3 hours an even higher increase in all activation markers was detected (CD11b(222%(137%-524%), CD11c(316%)). Neutrophil responsiveness to fMLF was most evident at T=0 and gradually decreased over time. Conclusion: Neutrophil activation significantly increased in the tube shortly after vena puncture particularly during the first hour. For a reproducible clinical test on neutrophil functionality it is mandatory to measure neutrophil receptor markers immediately after vena puncture in a point-of-care context.

P.E1.01.12

Identification of germline heavy chain targeted to envelope protein in acute phases of DENV2 secondary infected patients. N. Thammasonthijarern; Tropical Medicine, Bangkok, Thailand.

Identification of germline heavy chain targeted to envelope protein in acute phases of DENV2 secondary infected patients. N. Thammasonthijarern, W. Pourmameani, S. Benjathummakul, P. Ramsaoto, P. Patsakajakul
1 Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University, Thailand.
Dengue is one of the most important mosquito-borne viral diseases in humans, and lacking of vaccines and therapeutics contributes to disease burden. Dengue viral infection can cause a spectrum of clinical symptoms ranges. The understanding of human immunity creating antibody response that showed protection against infectious agent can be applied for vaccine design strategy. Antibody heavy chain genes generated at acute phase of secondary DENV-2 infected patients were studied from both human monoclonal antibodies (HuMabs) targeted to enveloped (E) proteins by clonal sequencing, and sample of B cell repertoires by next generation sequencing. In addition, antibody neutralizing activity was tested for foci reduction neutralization test (FRNT). Germline sequencing were analysed either by IMGT/V-Quest or IMGT/High5-Quest online program. Two major germline genes derived from 25 cross-neutralizing HuMabs were IGHVI-69 and IGHV3-23. Accordingly, IGHV6-69 is also the majority (32%) of antibody heavy chain genes derived from PBMC of acute phase dengue type 2 infected patient. It was implied that IGHV6-69 type of heavy chain was rapidly generated after DENV-2 infection at the acute phase and showed cross-neutralizing activity to 4 serotype and showed cross-protection in dengue patients. The result obtained from this study will be understanding for human antibody response correlated with further dengue vaccine design.

KEY WORDS: Dengue, PBMC, HuMabs

P.E1.01.14

Identification of small molecule compounds modulating the formation of neutrophil extracellular traps (NETS) P. Habenberger, P. Wentker, R. Di Lucerezi, G. Sollberger, M. Bickle, J. Eichkoff, A. Zychlinsky, R. Nussbaumere, M. B. Kleinb; Lead Discovery Center GmbH, Dortmund, Germany, 2 Max Planck Institute for Infection Biology, Berlin, Germany, 3 Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Formation of neutrophil extracellular traps is a non-reversible process of subsequently appearing, distinct cellular morphological features, which includes the loss of the characteristic lobulated nucleus. Breakdown of the nuclear and cellular membranes finally leads to release of several granule proteins and DNA / chromatin forming the extracellular NET fibres. Normally functioning as defence mechanism against extracellular pathogens, NET formation was also shown to be involved in the pathogenesis of several infectious diseases, sepsis, chronic lung disease, thrombosis and autoimmune disorders (e.g. systemic lupus erythematoses). Aim of potential therapeutic applications, we provide proof of concept in silico drug discovery. Objective was to use an in silico drug targeting the immunoglobulin light chain (FLC) that activates mast cells, on the outcome of an inflammation. Primary human neutrophils from healthy donors were stimulated with phorbol 12-myristate 13-acetate (PMA) and treated with ~180.000 compounds. After staining the DNA and the characteristic lobulated nucleus. Breakdown of the nuclear and cellular membranes finally leads to release of several granule proteins and DNA / chromatin forming the extracellular NET fibres. Normally functioning as defence mechanism against extracellular pathogens, NET formation was also shown to be involved in the pathogenesis of several infectious diseases, sepsis, chronic lung disease, thrombosis and autoimmune disorders (e.g. systemic lupus erythematoses). Aims of potential therapeutic applications, we provide proof of concept in silico drug discovery. For each species in the network, a balance equation was then formulated, specifying its time dependence as the differences between the rates of the reactions synthesizing the commodity and the rates of consumption. The corresponding rate equations were integrated in time or for steady state by using the COPASI simulation software. Immunostaining on cancer biopsies, protein detection, and sample of B cell repertoires by next generation sequencing. In addition, antibody neutralizing activity was tested for foci reduction neutralization test (FRNT). Germline sequencing were analysed either by IMGT/V-Quest or IMGT/High5-Quest online program. Two major germline genes derived from 25 cross-neutralizing HuMabs were IGHVI-69 and IGHV3-23. Accordingly, IGHV6-69 is also the majority (32%) of antibody heavy chain genes derived from PBMC of acute phase dengue type 2 infected patient. It was implied that IGHV6-69 type of heavy chain was rapidly generated after DENV-2 infection at the acute phase and showed cross-neutralizing activity to 4 serotype and showed cross-protection in dengue patients. The result obtained from this study will be understanding for human antibody response correlated with further dengue vaccine design.

KEY WORDS: Dengue, PBMC, HuMabs

P.E1.01.02

Visualizing immune responses - Part 2

P.E1.01.02.01

ACUTE AND CHRONIC INFLAMMATION AND MAST CELL ACTIVATION, IN SILICO A. Abdurkerim; VU University Amsterdam, Amsterdam, Netherlands.

Objective: An iterative process of model building is able to provide new understanding about the mechanisms of inflammation. We designed a computer model able to provide a rational explanation of the network's response to antigen in terms of acute or chronic inflammation. The model should calculate in silico the level of tumor necrosis factor alpha (TNF-α). It should likewise predict the effect of an in silico drug targeting the immunoglobulin light chain (FLC) that activates mast cells, on the outcome of an inflammation. Modelling: For each species in the network, a balance equation was then formulated, specifying its time dependence as the differences between the rates of the reactions synthesizing the commodity and the rates of consumption. The corresponding rate equations were integrated in time or for steady state by using the COPASI simulation software. Immunostaining on cancer biopsies, protein detection, and sample of B cell repertoires by next generation sequencing. In addition, antibody neutralizing activity was tested for foci reduction neutralization test (FRNT). The model reproduced experimental findings and was subjected to tests for dynamic stability and to sensitivity analysis. Conclusions: A systems mechanism for the effects of therapeutic peptides against cancer-associated inflammation was identified and make computable in terms of personalized molecular properties. This should facilitate further testing as well as provide a platform for personalized immunology.

P.E1.01.02.02


Current reagents such as peptide specific MHCII antibodies do not allow to prove peptide-MHCII dynamics during live interactions in vivo as they also block T cell- APC interactions. To address this, we aimed to design fluorescent antigenic peptides. Typically, fluorescent protein tags such as green fluorescent protein (GFP) consist of more than 200 amino acids thus have the potential of disrupting the conformation and functionality of the antigenic peptides. Therefore, we constructed an OVA<sub>323-339</sub> peptide containing a pro-fluorescent tetracycline tag (CCPCCG) at the C-terminus with aminocyclopropanic linker (OVA<sub>323-339</sub>). First, we tested whether enrichment of tag had an impact on T cell stimulation and found that OVA<sub>323-339</sub> could activate OTII cells to same degree as OVA<sub>323-339</sub>-MHCII as neither MHCII<sub>323-339</sub> produced same fluorescence. We also tracked the fluorescence in OTII- DC culture in vitro and detected that it got concentrated at the puncture in a point-of-care context.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 513
Inhibiting T cells establish immunological synapses forming cSMAC preferentially with MHCII antigen presenting cells in human GMBM.


Glioblastoma multiforme (GBM) is the highest and most aggressive grade of gloma, the most common primary brain tumor. Currently it remains incurable, as there are no effective treatments; however, the presence of an inflammatory environment characterized by immune cell infiltration and microglial activation, has suggested that its manipulation could represent a potential therapeutic strategy. In this study, immunological engagements between inhibiting T cells with tumor cells and microglia/macrophages were analyzed in detailed. Our results show that 70% of T cells establish physical appositions with either tumor cell subsets or MHCII+ cells, and that 55% of contacting T cells form features of immunological synapses, being much more likely to do so with MHCII+ cells, since the number of TCR rich supramolecular activation clusters (cSMACs) formed with these cells doubled the cSMACs formed with tumor cells. Importantly, we demonstrate that antigen presentation mediated by MHCII takes place in the tumor parenchyma and not only in the lymph nodes, suggesting that the brain tumor could be acting as a tertiary antigen presenting tissue to downregulate the cytolytic immune response. Furthermore, we believe that the IS formation between T cells and MHCII cells may be an strategy of the tumor to escape the immune response (i.e activating antigen-specific Tregs), so the inhibition of these engagements could represent a new immune checkpoint to block for potential immunotherapy in GBM.

POSTER PRESENTATIONS

P.E1.01.02
Real-time migratory pattern monitoring of neutrophil and macrophage toward skin melanoma microenvironment using two-photon intravital microscopy

Y. Choe, S. Jeong, Y. Kim, Y. Hyeon; Department of Anatomy and Brain Research Center 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea, Republic of.

Recent studies revealed that recruitment of myeloid cells toward tumor microenvironment can accelerate tumor progression during tumor development. However, the initial response of neutrophils and macrophages interacting with tumor cells in melanoma site has been not thoroughly visualized. Hereby, we investigated the crossstalk between metastatic skin melanoma and innate immune cells including neutrophils and macrophages. To directly visualize migratory pattern of neutrophils and macrophages toward melanoma microenvironment, we used set up skin window chamber for two-photon intravital microscopy. In order to investigate dynamic behavior, CMFDA (green) or CMTX (red) fluorescent probe-labeled murine melanoma cells, B16F10, were subcutaneously injected. To visualize neutrophils and macrophages, we used Ly6G-GFP and Ly6C-1 GF mice, respectively, as well as fluorescence. In this study, we will visualize each step of neutrophil infiltration toward melanoma cells at the early stage of subcutaneous injection of B16F10, and also demonstrate how to control the adhesion molecule molecular developmental endothelial locus-1 (Del-1) affects the migration pattern of neutrophils. We hypothesized that Del-1 deficiency may be involved in the extravascular migration of neutrophils in the presence of B16F10 melanoma cells. However, it was supposed that absence of Del-1 may not affect the interstitial migration of neutrophil to the tumor site. In this study, we will show the effect of Del-1 in neutrophil migratory cascade toward to melanoma microenvironment in crossstalk with resident macrophages in the early phase of innate immune response.

P.E1.01.05
A comparative analysis of targeted transcriptomic techniques and immunohistochemistry for immuno-oncology biomarker profiling in humanized mice

M. Houtkamp, N. Penscheva, F. de Bree, P. Franken, S. Verploegen, D. Schuurhuis, J. Lammers van Bueren; Gennab BV, Utrecht, Netherlands.

Traditional transcriptomic profiling techniques, such as RNA sequencing and microarrays, require relatively large sample input and their application on formalin-fixed paraffin-embedded (FFPE) samples is often hindered by RNA degradation. NanoString and HTG EdgeSeq are novel technologies that measure messenger RNA (mRNA) levels in a targeted fashion and can be applied to low amounts of FFPE samples for biomarker discovery. The ability of these targeted transcriptomic approaches to capture immune-related changes, as validated by immunohistochemistry (IHC), was assessed in human hematopoietic stem cell-reconstituted mice containing subcutaneous human tumors treated with a T-cell activating agent versus control. In addition, the NanoString PanCancer Immune Profiling Panel and the HTG immuno-oncology (IO) assay were compared for their concordance. Both NanoString (n=9) and HTG (n=12) generated data were observed. Both techniques identified a largely overlapping set of transcripts modulated upon treatment with T-cell activating agent. Importantly, both NanoString and HTG EdgeSeq mRNA measurements were further correlated with quantitative IE protein measurements for a selected panel of IO biomarkers modulated by T-cell activating treatment. In conclusion, both NanoString and HTG EdgeSeq are suitable technologies for transcriptomic assessment of IF biomarkers in FFPE samples. T-cell activating agent-induced transcriptomic changes, as assessed by either technology, are representative of immune alterations found by IHC - the current gold standard in the IO field.

P.E1.02.06
Identification of apoptotic cells and cells carrying extracellular vesicles during LCMV infection using imaging flow cytometry

J. Kranich1, N. Chis1, A. Leth1, L. Rausch1, F. Theis1, T. Bracker1; 1Institute for Immunology, 82152 Planegg-Martinsried, Germany, 2Heilmannzentrum München, 85764 Neuerberg, Germany.

Exposure of the phospholipid phosphatidylserine (PS) on the outer surface of the cell membrane is common to both, apoptotic cells and extracellular vesicles (EVs). Administration of a fluorescent version of the PS-binding protein Mfge8 (Mfge8-eGFP) in vivo in combination with imaging flow cytometry, allowed us to reliably detect and separate dying cells from living cells by EV-decorated live EVs. To discriminate both classes of Mfge8-eGFP expressing cells we applied a deep learning algorithm. Using this novel approach, we were able to very clearly visualize frequencies of dying cells in an in vivo experiment. However, localisation of apoptotic cells was readily detected mainly on B cells. Upregulation of PS was found in late stage of cell death (Figure 1). Here, a combination of EV-decorated CD8+ T cells increased approx. 4-fold. Here, EV-binding was found to be mostly confined on CD8+ T cells as well as follicular and marginal zone B cells. In addition, the frequency of EV-decorated CD8+ T cells as well as follicular and marginal zone B cells. In addition, the frequency of EV-decorated CD8+ T cells increased approx. 4-fold. Here, EV-binding was found to be mostly confined on CD8+ T cells, whereas in CD8- T cells and CD4+ T cells, but almost absent on CD4+ T cells (CD44+CD62L+). Our data visualize and quantify the propensity of activated CD8+ T cells to bind EVs, opening new avenues for investigation of potential functional consequences. Our data demonstrate that Mfge8-eGFP is a valuable tool for simultaneous identification of dying and EV-decorated cells in situ.

P.E1.02.07
Quantifying murine myocardial infarction and immune cell infiltration via light sheet fluorescence microscopy

S. F. Merz1, S. Karsen1, L. Borremans1, P. Stock1, U. Henegden-Cotta1, T. Rassaf2, M. Gunzer2, M. Tetzlaff2; 1Institute for experimental Immunology and Medicine, University Duisburg-Essen, Essen, Germany, 2West German Heart & Vascular Center, Department of Cardiology & Vascular Medicine, University Hospital Essen, Essen, Germany.

Myocardial infarction (MI) is one of the most lethal medical diseases in Western industrialized countries. To investigate the underlying mechanisms and to determine effects of novel therapeutic approaches an accurate quantification of infarct sizes in experimental models is imperative. Current protocols utilize life imaging (MRI, echocardiography) or slice-based histological methods, which suffer from poor resolution or limited representation of the entire heart, respectively. Here, we present a novel light sheet fluorescence microscopy (LSFM) approach for the precise quantification of relevant infarction model parameters including MI-size, area at risk (AAR) and heart volume in adult mice. Benefitting from ethyl cinnamate (EC) clearing, it allows simultaneous visualization of immune cells infiltrates in the whole heart at cellular resolution and can i.v. in vivo labeling of target structures utilizing i.v. injected conjugated antibodies and fluorochrome compatible buffering. Verifying its usefulness, we correlated our LSFM method with traditional TTC staining in an ischemia/reperfusion-injury mouse model. Here, TTC negative areas matched with capillary damage revealed by anti-C3b labeling of the endothelium in the same heart slices. Furthermore, we visualized macrophages and neutrophils via antibodies against F4/80 and Ly6-G, respectively. Neutrophils (24h reperfusion) were located in the vicinity, while macrophages (5d reperfusion) were localized within the infarction volume. Taken together, LSFM-based 3D quantification of infarction size represents a novel and precise tool to measure MI and to obtain crucial information about immune cell infiltration at the same time. This powerful approach enables multiplexing of various markers to investigate myocardial damage, infiltrating immune cells and their behavior.

P.E1.02.08
High throughput sequencing of TCR repertoire after yellow fever revaccination

A. A. Minervina, M. V. Popagrebo, E. A. Komech, I. Z. Mamedov, Y. B. Lebedev; Shemyakin–Ovchinnikov Institute of biorganic chemistry, Moscow, Russian Federation.

Introduction: Yellow fever vaccination is a well established model of acute viral infection in human. Primary immunization elicits strong T-cell response and the formation of long-lived memory. However, little data exists on the activation of this memory and individual T-cell clones dynamics after revaccination. We applied deep TCR-profilig to quantitatively track T-cell clones after yellow fever vaccination and revaccination. Methods: We isolated PBMCs from two donors -- one received primary vaccination, other revaccinated 30 years after -- on 5 timepoints: 0, 5, 10, 15, 45 days after YFV17D immunization. CD4+, CD8+, memory and MHC-dextrin positive subpopulations were collected on several timepoints. TCR repertoires were sequenced on illumina platform, expanded TCR clonotypes were identified using edgeR package.
Characterization of immune infiltrate and checkpoint protein expression patterns in murine syngeneic tumors via multiplex immunohistochemistry

M. Piekut, M. A. Karreman, A. S. Berghoff, K. Gunkel, W. Wick, F. Winkler; Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg; Clinical Cooperation Unit Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany.

Current knowledge on the immunological tumor microenvironment is gained from in vitro and ex vivo experiments, such as flow cytometry and immunohistochemistry. However, these models fail to recapitulate the dynamic influx of lymphocytes and their interaction with tumor cells in the complex tumor microenvironment. Particularly in brain cancer, which develops in an "immune-privileged organ", a better understanding of lymphocyte recruitment, trafficking and activation in vivo is urgently needed to improve immunotherapeutic approaches. To characterize the adaptive immune response in brain tumors on a single cell level, we developed a unique model allowing us to monitor these processes in vivo over weeks using repetitive two photon intravital microscopy. Hereeto, mice with a chronic cranial window were introduced with fluorescent syngeneic melanoma cells via heart injection or stereotactic injection into the cortex. Next, these mice received adoptive cell transfer of tumor-specific, fluorescently labeled T cells. We observed tumor-specific T cells crossed the brain blood barrier within a couple of hours. Notably, transferred T cells were found in the tumor margin and inside the tumor but not in healthy brain parenchyma. Furthermore, we could track lymphocytes at the site of the tumor for several days to explore their interactions with tumor cells. Taken together, we established a novel preclinical model to study dynamic lymphocyte-tumor cell interaction in vivo at high resolution. This approach provides unique insights into the underlying mechanisms driving the immune response. Importantly, it enables to further investigate and improve immunotherapeutic approaches with respect to prevention and therapy of brain metastases.

P. E1.02.09
Visualizing tumor cell-lymphocyte interactions in the brain using in vivo two photon microscopy

M. Piekut, M. A. Karreman, A. S. Berghoff, K. Gunkel, W. Wick, F. Winkler; Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg; Clinical Cooperation Unit Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany.

Characterization of immune infiltrate and checkpoint protein expression patterns in murine syngeneic tumors via multiplex immunohistochemistry

P. E1.02.10
Alteration of TCR repertoire after tick-borne encephalitis infection in murine model

M. A. Salnikova1,2, A. A. Minerinvina, V. M. Pogorelyj, K. K. Tuchinskaya, G. K. Garganova, Y. B. Lebedev, I. Z. Mamedov2; Shemyakin-Ovchinnikov Institute of Bioorganic chemistry, Moscow, Russia Federation; 1Moscow State University, Moscow, Russia Federation; 2Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russian Federation.

Introduction: Adaptive immune system is a very sophisticated mechanism, which is able to recognize great diversity of antigens. T cells play key role in adaptive immune response to tick-borne encephalitis virus. Deep TCR repertoire profiling can be performed via high-throughput sequencing. Murine models to study alterations in TCR repertoires during immune response are lacking. Here we present the method for longitudinal TCR repertoire reconstruction which identifies TCR clonotypes expanded in response to acute infection.

Materials and Methods: Peripheral murine blood was collected in two biological replicates before and after the infection with tick-borne encephalitis virus. TCRbeta cDNA libraries were prepared using custom protocol. Libraries were sequenced on Illumina platform. TCR repertoires were extracted from data using MiGEc and MiXCR software.

Results: We show good reproducibility of individual clones concentrations between biological replicates. edgeR package was used to identify significantly expanded or contracted after infection. We found 38 clones expanded and 1 contracted in response to viral infection. Most expanded clonotypes were not detected before infection, but were very abundant after, accounting for 6% of repertoire with 13 responding clones in top 20 clonotypes after the infection. Identified expanded clonotypes show considerable V usage bias and little overlap with published tick-borne encephalitis virus specific TCRbeta sequences.

Conclusion: This approach can be used to identify statistically significant changes in murine TCR repertoire during infections, vaccinations and drug tests. Supported by RSF15-15-00178.

P. E1.02.11
In vivo deuterium labeling of circulating immune cells: a feasibility study

E. Veld, in 't, H. W. Groeneveld, M. Balu Pique, J. Drylewicz, F. E. Stuurman, T. Kolk, van der, G. J. Groeneveld, J. A. Borghans, K. Tessler1, M. Moerland1; 1Centre for Human Drug Research, Leiden, Netherlands, 2UMCU, Utrecht, Netherlands.

Quantification of the life span of immune cells in vivo by deuterium labeling may be an attractive methodology when studying disease or drug effects. It requires cell isolation, which is operationally challenging in clinical studies. Moreover, limited data are available on the variability of cellular lifespan between subjects, and the correlation with cell phenotype. Herefore, a feasibility study was performed in 16 volunteers (8 healthy subjects/HV, and 8 MS patients). Subjects received 70% deuterated water for 9 weeks and were followed for one year. Immune cell subsets were isolated by sequential magnetic sorting (RoboSep), phenotyped (MACSQuant10), and analyzed for deuterium incorporation (GCMS) allowing calculation of life span.

Cell count strongly influenced cell purity. For CD19+ B-cells and CD4+ T-cells, average purities were 89 and 87% (CD4+, HV and MS) and 90 and 89% (CD19+, HV and MS). Cell counts were generally exceeding 30x10^6/mtm. However, for memory and naive CD8+ T-cells, average purities were 76 and 59% (CD8+m, HV and MS) and 73 and 68% (CD8+n, HV and MS), with counts below 15x10^6. Average life span was 271±232 days for CD19+ cells, and 209±283 days for CD4+ cells (minimal purity 85%), not clearly correlating with circulating cell numbers. Although the availability of automated sorters greatly facilitates cell sorting in clinical studies, our data demonstrate that the quality of the cells remains an important point-of-attention for customized isolation protocols. Life span of immune cell subsets was highly variable between subjects, complicating its use as readout in future clinical pharmacology studies.

P. E1.02.12
In vitro three dimensional vascularized skin on a chip to study of immune response

S. Kim; RIST, Seoul, Korea, Republic of.

Various kinds of human organ on a chip studies have been performed to screen the toxicity and efficacy of certain materials including drugs by various limitations of animal experiments such as the ethical and regulatory issues and the considerable difference between animals and human. Especially, skin on a chip is one of the studies that has attracted a lot of attentions because it can be used for screening of cosmetics and skin detergents as well as drugs. However, most of current in vitro skin models are based on two dimensional culture of fibroblasts and keratinocytes that only simulate human epidermis and dermis. One of the most serious problems among various skin reactions caused by chemical substances and biological agents, including drugs, cosmetics and skin detergents is inflammatory skin disease. To confirm this reaction, the vascular network structures, which regulate immune response, are need as well as the epidermis and dermis structures. For this reason, we investigated the in vitro three dimensional vascularized skin on a chip to mimic both structures and functional responses of the human skin using collagen/fibrin hydrogel (CHI) for dermis and poly(lactide-co-caprolactone)[PLCL] nanofibrous electrospun membrane(NEM) for epidermis. To mimic vessels, we created channels in the CHI and coated with human umbilical vein endothelial cells(HUVECs). Consequently, we could find that channels coated with HUVECs formed a vessels-like structure, and microvascular networks were formed in CHI like the dermis. Moreover, it was confirmed that PCL-NEM formed the epidermis like structure.

P. E1.02.13
Characterization of immune infiltrate and expression pattern of protein marker in murine syngeneic tumors via multiplex immunohistochemistry

J. Bewsher1, J. Kelly2, S. Kisi, K. K. Tuchinskaya, G. J. Groeneveld2, 1Cell Signaling Technology, Leiden, Netherlands, 2Cell Signaling Technology, Danvers, United States.

Murine syngeneic tumors models are increasingly utilized for preclinical immuno-oncology studies as immunotherapeutic strategies continue to make clinical strides. However, the immunologic features of the tumor microenvironment (TME) in these models remain largely undefined. In this study, we applied a 7-color multiplex immunohistochemistry (mIHC) panel to visualize and quantify the immune infiltrate within formalin-fixed, paraffin-embedded (FFPE) Renca, CT26, and LLC/L2 tumor tissues derived from subcutaneous mouse models of renal cell carcinoma, colon carcinoma, and lung carcinoma, respectively. Additionally, we applied this panel to a 4T1 orthotopic mammary tumor and 4T1 lung metastasis for further validation. This multiplex panel included antibodies detecting CD3, CD8 as T cell markers, F4/80 as a myeloid cell marker, the immunosuppressive receptor PD-1, as well as its ligand PD-1, pan-keratin as a tumor mask, and DAPI as a nuclear counterstain. We characterized the localization of tumor-infiltrating immune cells, as well as trends in the coexpression and frequency patterns of these proteins. This study strives to better understand the underlying differences in the immunologic landscapes of these tumors, which in turn has implications for researchers studying responses to immunotherapeutic approaches and combination strategies in murine models of cancer.
P.E1.02.14 REALEASE™ immunomagnetic separation technology with reversible labeling for positive selection of leukocytes


Immunomagnetic enrichment of leukocytes is an important technique in both research and clinical applications. Current enrichment strategies using magnetic labeling of the target cells allow for highly efficient isolation, but in some instances removal of residual cell surface labeling after isolation is of high interest. To this end, we have combined the benefits of positive selection by MACS® Technology, the proven state-of-the-art method for the clinical isolation of functional, viable cells, with a novel technology enabling the removal of both immunomagnetic beads and antibody fragments. REALEASE™ Technology provides an easy and fast solution for the highly specific isolation of unlabeled leukocytes directly from PBMCs. Separation based on this technology results in high purities of e.g. CD3+, CD4+, CD8+, CD19+, and CD56+ cells of around 95%. Here, we present our latest results on cell separation with REALEASE Technology validating activation, proliferation and re-labeling of important cell subsets such as CD4+ and CD8+ T cells, as well as CD19+ B cells. Target cell isolation did not induce cell activation while upon stimulation the cells were properly activated indicating preserved cell physiology.

P.E2.01.04 How to handle big data?

P.E2.01.01 Biostatistics - Association Between Age, Occupation & Morbidity, using publicly available NIH longitudinal study data

S. H. Cho; -, -, United States.

According to the NIH longitudinal study data publicly available, there has been a significant association between Age, Occupation and Profession. It has been found that those who were working as Professionals & in Related Occupations, has been found to be recorded as dead in their late 20s, while occupations such as Service Occupations, as well as people in Office & Administrative Support Occupations, seem to have seconded the high mortality rate. Farming, Fishing, and Forestry seem to have the least number of people in the public death records. It might mean that people in these occupations were never in the public death records or there had been least number of deaths in these occupations.

Acknowledgements - Harvard Medical School: Global & Continuing Education The National Longitudinal Mortality Study is a collaborative effort between the US Census Bureau and the National Heart, Lung, and Blood Institute (NHLBI), National Cancer Institute (NCI), National Institute on Aging (NIA), and the National Center for Health Statistics (NCHS).

The views expressed in this paper are those of the authors and do not necessarily reflect the views of the Census Bureau, NHLBI, NCI, NIA or NCHS.

P.E2.01.02 On the feasibility of mining CD8+ T cell receptor patterns underlying immunogenic peptide recognition

N. De Neuter1, W. Bittremieux1, C. Beirnaert1, B. Guypers1, A. Mirzai1, P. Moris1, A. Sois1, V. Van Tendeloo2, B. Oguinjmi3, K. Laukens1, P. Meysman2;
1Adrem Data Lab, Antwerpen, Belgium, 2Center for Medical Genetics, Antwerpen, Belgium, 3Laboratory of Experimental Hematology, Antwerpen, Belgium, 4Centre for Health Economics Research & Modeling Infectious Diseases Lab, Antwerpen, Belgium.

Current T cell epitope prediction tools are a valuable resource in designing targeted immunogenenity experiments. They typically focus on, and are able to, accurately predict peptide binding and presentation by major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells. However, recognition of the peptide-MHC complex by a T cell receptor is often not included in these tools. We developed a classification approach based on random forest classifiers to predict recognition of a peptide by a T cell and discover patterns that contribute to recognition. We considered two approaches to solve this problem: (1) distinguishing between two sets of T cell receptors that each bind to a known peptide and (2) retrieving T cell receptors that bind to a given peptide from a large pool of T cell receptors. Evaluation of the models on two HIV-1, B*08-restricted peptides reveals good performance and hints towards structural CDR3 features that can determine peptide immunogenicity. These results are of particular importance as they show that prediction of T cell epitope and T cell epitope recognition based on sequence data is a feasible approach. In addition, the validity of our models not only serves as a proof of concept for the prediction of immunogenic T cell epitopes but also paves the way for more general and high performing models. This research was funded by the University of Antwerp [BOF Concerted Research Action] and the Research Foundation Flanders (FWO) [Personal PhD grants to NDN (1529816N), PMo (1141217N), BC (1101614N)].

P.E2.01.03 TCReX: a webtool for the prediction of TCR-epitope recognition


To date, multiple immunoinformatics tools have been created with the goal to achieve a better understanding of immunological processes. Although great tools exist for the prediction of epitopes and their binding to MHC molecules, we are still lacking useful tools for the prediction of epitope-MHC recognition by TCRs. Hence, we propose TCReX, a tool to investigate TCR recognition of epitopes. This tool is based on our prior work related to the feasibility of predicting TCR-epitope recognition using TCReB sequences. In this study, we showed that a random forest classifier trained to predict TCR-epitope interactions from TCR amino acid physicochemical properties can achieve a high accuracy. We are extending this work into a toolbox trained on a large dataset containing epitopes from different viruses, such as HIV-1 and EBV, and tumour cells. To this end, we collected epitope-specific human TCRβ sequence data containing information about the CDR3 sequences and the corresponding V-and J-genes. Random forest classifiers are trained on this data and kept if they report a sufficiently high performance in a cross-validation setting. These classifiers will be made freely available in a webtool, called TCReX. TCReX will be useful to make predictions on newly gathered experimental TCReB sequence data. As such, it will aid researchers in the development of more specific epitope-TCR binding assays and the elucidation of T cell repertoire targets.

P.E2.01.04 IUPHAR Guide to IMMUNOPHARMACOLOGY

S. D. Harding1, E. Facenda2, A. J. Pawson3, J. L. Sharram1, C. Southan1, S. P. Alexander1, S. Anderson1, C. Bryant1, A. Davenport1, C. Doering1, D. Fabro1, F. Levi-Schaffer1, M. Spedding1, J. A. Davies1;
1University of Edinburgh, Edinburgh, United Kingdom, 2University of Nottingham, Nottingham, United Kingdom, 3University of Cambridge, Cambridge, United Kingdom, *University of Manchester University, Melbourne, Australia, **Pfizer Therapeutics, Basel, Switzerland, #Hebrew University of Jerusalem, Jerusalem, Israel, $Spyder Research Solutions, Le Vesinet, France.

Immunity, inflammation and infection have become priority areas in drug discovery research. Most chronic diseases, including ageing, have an immune-inflammatory component; auto-immunity is a serious problem, and the progress of infections depends on immune and inflammatory responses. The International Union of Basic and Clinical Pharmacology (IUPHAR) have developed a new online resource called the IUPHAR Guide to IMMUNOPHARMACOLOGY (GtoImmuPdb; www.guideimmunopharmacology.org) that aims to improve data exchange between immunology and pharmacology expert communities, to better support research and development of drugs targeted at modulating immune, inflammatory or infectious components of disease. Supported by the Wellcome Trust, GtoImmuPdb is an extension to the existing IUPHAR/BPS Guide to PHARMACOLOGY (GtoPdb; www.guidetopharmacology.org), a joint initiative between IUPHAR, the British Pharmacological Society (BPS) and the University of Edinburgh. It is an open-access and regularly updated resource, providing a unique access point that is user-friendly to immunologists and pharmacologists alike. Its integration with GtoPdb comes with enhanced search mechanisms and new ways to browse and visualize immunological data. Details of the expansion are provided in our recent update in the 2018 NAR and Disease paper (1). The data in the IUPHAR Guide to IMMUNOPHARMACOLOGY, and its parent database, are sourced from peer-reviewed primary literature and their content is curated by committees containing >500 global expert contributors.


Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands
POSTER PRESENTATIONS

P.E2.01.05
Computational search of active compounds for SH2

V. Hurmach1, A. Varnek1, M. Platov2, Y. Prylatskyj1.

1Taras Shevchenko National University of Kyiv, Kyiv, Ukraine, 2University of Strasbourg, Strasbourg, France, Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine.

Search for the new chemical structures with specific biological activity is a problem, which requires usage of the latest achievements in molecular modeling technologies. It is well known that SH2 domains are involved in intracellular signaling pathways. Their dysfunction is related with such cancer diseases as Basal cell carcinoma, T cell acute lymphoblastic leukemia, and other cell types. The aim of this work - investigation of SH2 domain binding properties and searching for new potential active compounds in the whole SH2 domains class. All available structures of SH2 domain were divided to six groups with high/average level of conservation (group 1 - 92%, group 2 - 78%, group 3 - 47%, group 4 - 32%, group 5 - 39%, group 6 - 45%) and not significant RMSD difference (0.8 Å, 1.16 Å, 1.6 Å, 1.9 Å, 2 Å, 1.4 Å).

Structure analysis showed that binding pocket contains 20-24 main amino acids: FLVRESETT (pTyr binding part), β-sheet; KYRK (central β), β-sheet; I5R (acidic pocket), BG-sheet and α-helix. Seven most representative pockets were selected based on those sequences, and used for docking and pharmacochemistry study of the entire Enamine Ltd database (> 1 M compounds). The outcome of this stage is selection of 10463 compounds. To conclude, specific scaffolds have been identified as pTyr substitutes. In almost all cases the ligand tightly fills the phosphotyrosine binding site and creates hydrogen bonds with the key amino acids Arg and Lys.

P.E2.01.06
REAfinity recombinant antibodies can be used for Immunofluorescence staining of fixed mouse and human tissues

A. Kinkhabwala1, W. Muller1, D. Rockel2.

Miltenyi Biotec GmbH, Bergisch Gladbach, Germany.

REAfinity™ recombinant antibodies are available from Miltenyi-Biotec conjugated to various fluorochromes. The main advantage of recombinant antibodies compared to hybridoma derived antibodies is the reproducibility of their production. Our research areas are the mammalian cell lines that are used as expression systems. One of the main challenges of this research area is the creation of antibodies that are used as multicolour immunofluorescence images from fixed cells or tissues. Here we show that REAfinity™ recombinant Antibodies can be used to stain various tissues and cells from mouse or human origin fixed by two fixation method and that they can be used in a multiplex manner. Examples of fixed tissue sections and single cell mixtures will be shown for a selected subset of REAfinity™ antibody staining demonstrating that REAfinity™ recombinant Antibodies can be used not only for flow cytometry but also for immunofluorescence staining of fixed cells and tissues.

P.E2.01.07
Robust prediction of peptide-MHC binding affinity with deep neural networks

V. I. Nazarov1, V. Tsvetkov1, E. Oftserov1.

1Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russian Federation, 2Pirogov Russian National Research Medical University, Moscow, Russian Federation, 3Tula State University, Tula, Russian Federation.

Current adaptive immune research pose significant challenges to data analysis. One of the major challenges toward personalized vaccine design and other applications is the prediction of peptide-MHC binding affinity. The major historic controversies come from their ligands. The emerging field of mass spectrometry immunopeptidomics complements existing binding affinity data, although common machine learning models are trained mostly on binding affinity data alone. Moreover, available data is often noisy and redundant. Therefore models inferred from this data can’t reach high accuracy due to data bias and corruption. In order to address both issues deep neural network was designed and trained on both binding affinity and mass spectrometry data. Specific architecture and learning process modifications were introduced to increase model robustness and to handle corrupt data. Altogether these advantages of designed deep learning algorithm allowed us to compete with state-of-the-art models, e.g. netMHCPan, and achieve comparable performance in at least 10 times less computation time.

P.E2.01.08
Identification of mutations associated with autoinflammatory diseases by Next Generation Sequencing

I. Olivas Martinez1, M. Montes Cona2, L. Gonzalez Garcia3, J. Bernabeu Witter4, L. Fernandez Silveira5, M. Camacho Lovilla6, Hospital Virgen del Rocio, Sevilla, Spain.

The aim was to evaluate a patient with inflammatory symptoms and mild immune disorders using Next Generation Sequencing (NGS). Patient is a 27-year-old man with chilblain lesions that appear in winter and recurrent febrile episodes who was subjected to biochemistry analysis, imaging techniques (Pulmonary CT scan) and autoimmunity studies (ANA, ANCA). A genetic analysis was performed using NGS with a panel of 12 genes related to autoinflammation diseases: NLRP1, TLR1/2/4, MVK, PSTPIP1, MVP, NOD2, CECD1, TMEM173, IL1RN, IL6RN, LPIN2 and NLRP12. Patient’s DNA was amplified by multiplex PCR and sequenced using Ion Torrent platform. Mutations identified were confirmed by Sanger methodology. Imaging and autoimmunity studies were negative, but the presence of characteristic lesions, hepatic enzymes increased, neutrophilia and lymphopenia, made clinicians suspect an autoinflammatory SAVI-like syndrome (STING-Associated Vasculopathy with onset in Infancy). Four heterozygous mutations associated with autoinflammatory disorders were found: in exon 9 of MEFV gene with amino acid change V81I, in exon 11 of MIF gene with change V377I, in exon 7 of LPIN2 gene with change A331S and in exon 5 of TMEM173 gene, producing the change F153V. All these mutations were classified as non-pathogenic by the statistical analysis tool PolyPhen-2. F153V had not been described before, being located next to a pathogenic mutation (N154S), so we can’t rule out its possible role in the above-mentioned pathology. Identification through NGS of 4 different mutations related to autoinflammatory syndromes in the same patient allows to extend the spectrum of mutations and phenotypes associated with these immune disorders.

P.E2.01.09
Novel miRNAs revealed in bovine macrophages stimulated with pathogen associated molecular patterns

F. N. Toku1, K. Smith1, L. Sout-Dabrowska1, M. B. Mielcarek1, P. Kielbasa2, M. Bossowska-Nowicka1.

1University of Veterinary Medicine, Bassesterre, Saint Kitts and Nevis, 2Faculty of Veterinary Medicine, SGWU, Warsaw, Poland.

Introduction: MicroRNAs influence biological processes during development, cell differentiation and infection. We have mimicked bacterial and viral infection with pathogen associated molecular patterns to assess the early phase expression of miRNAs in a bovine macrophage cell line Bomac.

Material and Methods: Cells were stimulated with CpG or poly(C:C) for 6 h, and miRNAs isolated using an enrichment protocol and sequenced in Illumina system. Data were analyzed in mirDeep2. Read counts for mature miRNAs were input into R for data pre-processing and statistical analysis using Bioconductor.

Results: The 9 samples had 18-31 million reads. miRNAs with 0.5 CPM in at least 3 samples were filtered out, leaving 1,318 miRNAs, comprised of 671 novel miRNAs, 564 known miRNAs that mapped to the genome and 83 known miRNAs that could not be mapped to the genome. Nine miRNAs were compared with the miRNAs; the overall FDR threshold was 0.35. Of these nine, seven were upregulated by CpG only and two were upregulated by poly(C:C) only. miRNAs negatively regulate their targets, we looked for miRNAs with -6 - 7% Pearson correlation coefficients between miRNA expression and mRNA expression. 580 miRNAs were negatively correlated with one or more of the 7 CpG miRNAs and 507 miRNAs were negatively correlated with one or more of the 2 poly(C:C) miRNAs. Nine novel miRNAs were validated by Taqman small RNA assays and found to be expressed at levels that correlated with sequencing data.

Conclusion: We have discovered novel bovine miRNAs that may influence gene expression in infected bovine macrophages.

P.E2.01.10
The prediction of specific antibody- and cell-mediated responses using baseline immune status parameters of individuals received measles-mumps-rubella vaccine.

C. Grebennikova1, A. Toptygina2, A. Bocharov3.

1Moscow Institute of Physics and Technology (State University), Moscow, Russian Federation, 2Marchuk Institute of Numerical Mathematics of the Russian Academy of Sciences, Moscow, Russian Federation, 3Lomonosov Moscow State University, Moscow, Russian Federation.

The successful vaccination implies the induction of effective specific immune responses. We intend to find biomarkers among various immune cell subsets, cytokines and antibodies which could be used to predict the levels of specific antibody and cell-mediated responses after measles-mumps-rubella vaccination. To do this, we measured 59 baseline immune status parameters (frequencies of 42 immune cell subsets, levels of 13 cytokines, immunoglobulins) before vaccination and 13 response variables (specific IgA and IgG, antigen-induced IFN-y production, CD107a expression, and cellular proliferation levels by CFSE dilution) 6 weeks after vaccination for 19 individuals. Statistically significant Spearman correlations between some baseline parameters and response variables were found for each response variable (p<0.05). Due to the low number of observations relative to the number of baseline parameters and missing data for some observations, the automatic stepwise procedure of minimal adequate multivariable linear regression selection without overparameterization is used. To reduce the number of candidate parameters, we manually selected for each response variable 5 parameters among the parameters which correlated with response variable with p<0.2, and which were as much as possible uncorrelated between each other. Given the manually chosen candidate parameters, we managed to identify the minimal adequate predictive multivariable linear regression models of post-vaccination antibody- and cell-mediated responses with up to 5 predictors in each model. The work is supported by the Russian Foundation for Basic Research (grant 17-19-00636).

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

517
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

POSTER PRESENTATIONS

P.E2.01.11
Exploring large-scale flow cytometry data for identification of immune-related genes
L. Treise1, Y. Cha1, G. Miller2, H. Fuchs3, V. Galius-Durner1, M. Hrabé de Angelis1, D. H. Busch2
1Institute of Experimental Genetics, München/Neuerberg, Germany, 2Institut für Medizinische Mikrobiologie, Immunologie und Hygiene, München, Germany.

Immune-related diseases such as autoimmune or inflammatory disorders are a common clinical problem, but the underlying genetic factors are not yet fully understood. A large-scale approach is required to characterize protein-coding genes, their functional evidence and their relevance for immune system-related diseases. The International Mouse Phenotyping Consortium (IMPC) aims at generation and phenotypic characterization of knockout mouse lines of all coding genes. Within the Immunology Unit of the German Mouse Clinic we systematically analyzed flow cytometry data of mice from the IMPC resource for abnormalities in the immune cell composition of the spleen. Multicolor flow cytometry is a powerful tool for simultaneous acquisition of a vast diversity of immune cell populations.

Components of both the innate and adaptive immunity were assessed: T, B, and NK lymphocytes and their respective subpopulations, as well as myeloid cell subsets, e.g. monocytes, granulocytes, macrophages and dendritic cells. Here, we show multiparametric analysis of large-scale flow cytometry data of more than 150 mutant mouse lines. We use statistical tools to visualize relations of the different immune cell subpopulations and to identify genes relevant for immune disorders.

P.E2.01.12
T and B cell repertoire sequencing: quality control and clone identification
B. D. C. van Schoik1, P. L. Kloorensbeek1, M. E. Doorenpleet1, S. Pollastria1, A. Musters1, G. Balarezo1, R. E. Essex1, F. Baas1, N. de Vries1, A. H. van Kampen1
1Academic Medical Center, Amsterdam, Netherlands, 2Leiden University Medical Center, Leiden, Netherlands, 3University of Amsterdam, Amsterdam, Netherlands.

Next-generation sequencing (NGS) of T and B cell receptor repertoires was introduced in 2009 as a new powerful tool in immunology research. Repertoire sequencing has found various applications such as the elucidation of plasmablast antibody subtypes for elucidating vaccine-induced antibody responses and cellular immunity. The major goal of BRepertoire is to facilitate easily accessible, fast and responsive data partition comparisons in the analysis of the antibody repertoire. A powerful data preparation pipeline and a set of analysis functionalities including PCA, V(D)J gene usage and statistical comparisons enables the investigation of repertoires in many different ways and future projects.

1Grants: This work was carried out on the Dutch national e-infrastructure with the support of SURF Cooperative. P.L.K. was funded by a PhD scholarship of the AMC Graduate School.

P.E2.01.13
Arbitrary transformation of flow cytometry data for multivariate analysis may produce misleading immunological information
S. van Stoveren1, R. Guittierre1, R. Folcarelli1, E. Cadot1, J. J. Jansen1, N. Vissergroep1, L. Koenderman1, O. F. van den Brink1
1UMC Utrecht, University, Netherlands, 2Radboud University, Nijmegen, Netherlands.

Introduction: The flow cytometry field has evolved rapidly, allowing the measurement of 30-50 parameters per cell. This has led to a tremendous increase in multivariate information. Manual gating is insufficient to extract all this information. Therefore, multivariate analysis (MVA) methods have been developed like cluster methods SPADE and FlowSOM and dimensionality reduction methods ViSNE, FLOOD, DAMACY and ECLIPSE. To aid interpretation, the data are often transformed logarithmically before MVA.

Rationale: We studied the consequences of different transformations of flow data in datasets containing negative intensities caused by background subtractions and compensation, as logarithmic transformation of negative data is not possible. Alternative transformations such as biexponential, or logistic, and hyperbolic arcsine transformations allow linearity around zero, whereas higher (positive and negative) intensities are logarithmically transformed.

Results: To define the linear range, a parameter (or co-factor) must be chosen. The type of transformation and the concomitant chosen parameter have great impact on the MVA results. In some cases, peak-splitting is observed, producing two distributions in an actual homogeneous population around 0. This may be misinterpreted as the presence of multiple distinct populations. We applied various statistical methods to optimally choose the parameter of the transformation used.

Conclusion: Arbitrary or unrecognized transformation can lead to wrong conclusions for cluster methods as well as dimensionality reduction methods. Importantly, it should be noted that some MVA methods only support one transformation option with a pre-set parameter in their algorithms. We recommend to transform flow cytometry data separately per channel, to prevent peak-splitting.

P.E2.01.14
Knowing what is what: Artificial Intelligence for cell classification in cytometry
J. Verhoeof1, J. J. Garcia-Vallejos1, S. Abehi1
1Cancer Center Amsterdam, Amsterdam, Netherlands, 2VU university Amsterdam, Amsterdam, Netherlands.

The advent of high parametric cytometry systems (10+ color flow cytometry and mass cytometry) has greatly increased the amount of data gathered from single cell suspensions. Classical gating mistakes go out on multi-parametric relations not visible on sequential biaxial plots, leading to the creation of computational tools, capable of clustering cells in an unsupervised manner. However the identified clusters would still need interpretation by a skilled immunologist to label them. This work details our strategy to develop a cell classifier suited for mass cytometry data. We hypothesize a supervised approach is preferable over the laborious process of identifying unknown clusters. By identifying a neural network logistical regression model based on softmax regression. Softmax regression is a generalized logistic function. This model took as input a small set of classically gated and labeled cells (500 of each label) from 1 sample per batch. Initial results indicate a success rate of >95% in training, test and validation sets. The abundance of subsets does not affect the performance of the model.

P.E2.01.15
FlowSOM and dimensionality reduction methods viSNE, FLOOD, DAMACY and ECLIPSE. To aid interpretation, the data are often transformed logarithmically before MVA.

Rationale: We studied the consequences of different transformations of flow data in datasets containing negative intensities caused by background subtractions and compensation, as logarithmic transformation of negative data is not possible. Alternative transformations such as biexponential, or logistic, and hyperbolic arcsine transformations allow linearity around zero, whereas higher (positive and negative) intensities are logarithmically transformed.

Results: To define the linear range, a parameter (or co-factor) must be chosen. The type of transformation and the concomitant chosen parameter have great impact on the MVA results. In some cases, peak-splitting is observed, producing two distributions in an actual homogeneous population around 0. This may be misinterpreted as the presence of multiple distinct populations. We applied various statistical methods to optimally choose the parameter of the transformation used.

Conclusion: Arbitrary or unrecognized transformation can lead to wrong conclusions for cluster methods as well as dimensionality reduction methods. Importantly, it should be noted that some MVA methods only support one transformation option with a pre-set parameter in their algorithms. We recommend to transform flow cytometry data separately per channel, to prevent peak-splitting.

P.E2.01.16
BRepertoire: A user-friendly webserver for analysing antibody repertoire data
D. Dunn-Walters1, C. Margreiter1, F. Fraternali2
1University of Surrey, Guildford, United Kingdom, 2King’s College London, London, United Kingdom.

The major goal of BRepertoire is to facilitate easily accessible, fast and responsive data partition comparisons in the analysis of the antibody repertoire. A powerful data preparation pipeline and a set of analysis functionalities including PCA, V(D)J gene usage and statistical comparisons enables the investigation of repertoires in many different ways and ultimately the identification of distinguishing features between sub-groups of data. As most functions provide plots in addition to the text results, a quick screening of the data and visual inspection of the results is also possible. We believe BRepertoire to be an invaluable tool for experimental immunologists who study immune repertoires. A unique functionality is flexible analysis of physico-chemical properties of amino acid sequences, moving towards a better understanding of receptor-antigen interactions. Since flexibility is induced in both the Treg and Tfh-like lineages upon activation, while CTLA4 is induced in the Tfh-like lineage only. We identify marker genes to distinguish these new populations.

P.E2.01.17
Single cell multidimensional analysis reveals activation-induced generation and maintenance of regulatory T cells during homeostasis and in tumour microenvironments
A. Bradley1, T. Hashimoto2, M. Ono1
1University of Tsukuba, Tsukuba, Japan, 2Department of Life Sciences, London, United Kingdom.

Background: T cell receptor (TCR) signalling initiates downstream transcriptional mechanisms for T cell activation and differentiation. FoxP3-expressing regulatory T cells (Treg) require TCR signals for their suppressive function and maintenance in the periphery. It is, however, unclear how TCR signalling controls the transcriptional programme of Treg, and whether it is conserved and how Treg are generated from activated T cells. Results: Here we dissect the transcriptions of various T cell subsets using multidimensional and single cell analysis methods. We show that Treg are as activated as memory-phenotype T cells (1mem) and effector T cells (Teff) at the transcriptomic level, and identify the common activation-dependent gene modules for these T cell subsets. Importantly, the major feature of Treg is that FoxP3 represses Runx1-associated, 1mem-specific activation-dependent genes, while FoxP3 sustains or enhances the expression of the common activation genes. Furthermore, by analysing single cell RNA-seq data of tumour-infiltrating T cells from melanoma patients, we show that activated T cells in the tumour microenvironments dynamically differentiate into two lineages of: Treg and T follicular helper (Thf)-like cells. We identify the bifurcation point of Treg and Thf-like differentiation, which specifically includes IL2-producing cells. Notably, CTLA4 is induced in both the Treg and Thf-like lineages upon activation, while PD1CD27 is induced in the Thf-like lineage only. We identify marker genes to distinguish these new populations.
POSTER PRESENTATIONS

P.E3E4.01 From single cells to population dynamics: memory T cell communication and signaling in the immune system

J. J. A. Calis1,2, A. A. Uh1, R. Carro1, B. R. Rosenberg1.

1The Rockefeller University, New York, United States, 2Icahn School of Medicine at Mount Sinai, New York, United States.

Introduction. An effective immune response relies on the coordinated activity of many cell types and associated functions. Transcriptomics methods enable detailed characterisation of these complex and dynamic processes. RNA-Seq and microarray studies of peripheral blood samples from human volunteers have revealed numerous gene expression networks and corresponding cellular functions that contribute to effective immunity. Recently developed high-throughput single cell RNA-Seq (scRNA-Seq) technologies offer great potential for further characterization of immune function at increased resolution. In measuring gene expression in individual cells, these methods can resolve different expression networks and corresponding cellular functions that contribute to effective immunity. Recently developed high-throughput single cell RNA-Seq (scRNA-Seq) technologies offer great potential for further characterization of immune function at increased resolution. In measuring gene expression in individual cells, these methods can resolve different expression networks and corresponding cellular functions that contribute to effective immunity.

Results. Preliminary results include a robust interferon response at days 3 and 7 post-vaccination, with distinct interferon-stimulated gene (ISG) expression programs induced in different cell types. At later time points, we observed activated T and B cell expansions characteristic of the adaptive immune response to vaccination. Additional analyses have revealed numerous cell type-specific gene expression programs in the response to this highly effective and clinically significant vaccine.

P.E3E4.01.01 Dietary lipids can modify the immune-related transcriptome in Atlantic salmon: a model organism

M. Jalili1,2, I. Mikelez1,2, S. Otto1, L. Borkner1, I. Terrén1,2, A. Bones1, M. Gerdel3, I. Denicola1, S. Otto1, L. Borkner1, I. Terrén1,2, A. Bones1, M. Gerdel3.

1CIC biomaGUNE, Donostia-San Sebastián, Spain, 2Ikerbasque, Basque Foundation for Science, Bilbao, Spain, 3Basque Centre for Genetic Engineering Research, Donostia-San Sebastián, Spain.

Abstract. Dietary lipids can modify the immune-related transcriptome in Atlantic salmon: a model organism

Description. Dietary lipids can modify the immune-related transcriptome in Atlantic salmon: a model organism

Background. Dietary lipids can modify the immune-related transcriptome in Atlantic salmon: a model organism

Introduction. Dietary lipids can modify the immune-related transcriptome in Atlantic salmon: a model organism

Methods and Materials. After 12 weeks at aquaculture facility, pyloric caeca (PC) and liver (LV) tissues were dissected from fishes and RNA was extracted. cDNA libraries were prepared and sequenced according to the next-generation sequencing protocol. The raw data were processed, mapped, quality-checked and analyzed statistically to compare different sampling time points (Day0, Day48 and Day94) and feedings (FO, VO and PL) in two types of tissues (PC, LV) in order to determine the significantly up- and down-regulated immune-related genes and their involvement in the expression of immune receptors (C1QTNF and C2D2), as well as the expression of CDS and IL-2-induced proliferation. Furthermore, we have checked the effects of the Jak1/2 inhibitor ruxolitinib in the generation of CML NK cells. We found that ruxolitinib-treated CML NK cells express lower levels of CD25 than non-treated CML NK cells, but show similar IL-2-induced proliferation. Moreover, we have also found that ruxolitinib-treated NK cells show reduced effector functions after the preactivation, and the CML NK cells could be recovered after 30% of the low doses of IL-2. Altogether, our results describe the impact that each cytokine and the Jak1/2 pathway have in the phenotype, IL-2 induced proliferation and effector function of human CML NK cells. Funding: AECC-Spanish Association Against Cancer (PROY1610748OR) and BIOEF-Basque Foundation for Research and Innovation-EITB Maratoia (BIO14/TIP/003).

P.E3E4.01.02 Role of interleukin (IL)-12/15/18 and ruxolitinib in the phenotype, proliferation and polyfunctionality of human cytotoxic preactivated NK cells

I. Terrén1, I. Mikelez1,2, I. Orajolo2, A. Geddllo3, I. González2, I. Arronta2, D. Zennuruzzabaloi2, J. Vidal3, F. Borrego4,5.

1Biocruces Health Research Institute, Barakaldo, Spain, 2CIC biomaGUNE, Donostia-San Sebastián, Spain, 3Warbasque, basque Foundation for Science, Bilbao, Spain, 4Basque Centre for Genetic Engineering Research, Donostia-San Sebastián, Spain.

Abstract. IL-12/15/18 and ruxolitinib have been implicated in the phenotype, proliferation and polyfunctionality of human cytotoxic preactivated NK cells

Background. IL-12/15/18 and ruxolitinib have been implicated in the phenotype, proliferation and polyfunctionality of human cytotoxic preactivated NK cells

Introduction. IL-12/15/18 and ruxolitinib have been implicated in the phenotype, proliferation and polyfunctionality of human cytotoxic preactivated NK cells

Methods. Human CD8+ T cells from three different donors were activated for 4 days with IL-12 (40 ng/mL), IL-15 (100 ng/mL) and IL-18 (100 ng/mL) in combination and the effect of ruxolitinib (1 and 5 μM) on the cytokine response was determined by using ELISA. The proliferative effect of the cytokines and ruxolitinib were determined by using 3H-thymidine incorporation assay. The effector functions of the cytokines and ruxolitinib were determined by using a tetramer staining assay and a chromium release assay.

Results. The combination of IL-12/15/18 cytokines significantly increased the expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms. Perforin mediated primary necrosis and slower apoptotic events were of less importance for both subpopulations. Expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms. Perforin mediated primary necrosis and slower apoptotic events were of less importance for both subpopulations. Expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms. Perforin mediated primary necrosis and slower apoptotic events were of less importance for both subpopulations. Expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms. Perforin mediated primary necrosis and slower apoptotic events were of less importance for both subpopulations. Expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms. Perforin mediated primary necrosis and slower apoptotic events were of less importance for both subpopulations. Expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms. Perforin mediated primary necrosis and slower apoptotic events were of less importance for both subpopulations. Expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms. Perforin mediated primary necrosis and slower apoptotic events were of less importance for both subpopulations. Expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms. Perforin mediated primary necrosis and slower apoptotic events were of less importance for both subpopulations. Expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms.

Conclusion. Using a high-resolution immune cell cytotoxicity assay on single cell level sheds light on the kinetics, efficiency, and mode of TCM or TTE induced target cell lysis in detail.
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

P.E3E4.01.06
Comparative analysis of transcriptomic data obtained from SCLC cells upon IFN-gamma response display great heterogeneity.

A. M. KURSNE1, E. Z. TASKIRAN2, G. ESENADAGL1;
1Department of Basic Oncology, Hacettepe University Cancer Institute, Ankara, Turkey; 2Department of Medical Genetics, Hacettepe University Faculty of Medicine, Ankara, Turkey.

Introduction: Small cell lung cancer (SCLC) which comprises 13% of all lung cancer cases is associated with aggressive tumor growth together with a tendency to early dissemination and distant metastasis. IFN-g is the uppermost cytokine implicated in anti-tumor immunity. Although it plays a central role in the recognition and elimination of transformed cells, many immune regulatory pathways can also be induced. The aim of this project is to determine whether an IFN-g-related immune activation occurs against SCLC cells and whether such SCLC cells come with anti-tumor effects of IFN-g. Materials and Methods: SCLC cell lines were co-cultured with PBMCs. T-cell proliferation, IFN-g and IL-2 secretion were determined. Additionally, SCLC cell lines were exposed to recombinant IFN-g and total RNA was extracted. RNA-seq library preparation and next-generation sequencing (NGS) were performed. NGS data were analyzed using various approaches (STRING, KEGG). Results: SCLC cells promoted the proliferation of T-cells within the PBMCs. IL-2 and IFN-g levels were also elevated in supernatants collected from the SCLC/PBMC co-cultures. Differential effects of IFN-g on each SCLC cell line was observed. Especially major immune regulatory pathways (such as PD-L1 and IDO1) were heterogeneously modulated. Discussion: These data indicate an adaptive resistance mechanism for SCLC since these cells initially attract T-cell activation and IFN-g secretion. SCLC cells are responsive to IFN-g while different immune regulatory pathways were induced. Thus, exposure to IFN-g revealed alternative mechanisms employed by SCLC cells to cope with immune responses. These data might provide new insights for cancer biology and immune therapy treatments.

P.E3E4.01.07
Characterising the functional and genomic profile of CAR CD19 T cells using single cell analyses

F. Luciani1, L. Clancy1, C. Caï1, H. McGuire1, E. Koshekerian1, D. Kappstein2, E. Blyth2, D. Gottlieb1, K. Mickelthwait1; 1School of Medical Sciences, Sydney, Australia, 2Westmead Institute for Medical Research, University of Sydney, Sydney, Australia.

Despite the great success of CAR T-cells in the treatment of blood malignancies the mechanisms underpinning long-term persistence and limited toxicity remain unknown. The presence of less-differentiated T-cells such as T-stem-cell memory (TSM) have been correlated to long-term persistence and limited toxicity. A single cell (sc)RNAseq approach has been developed to identify and track post-infusion the transcriptional signatures and endogenous TCR of CAR-T cell subsets and to link these profiles to clinical data. Three CAR T-cell products have been administered with complete molecular remission in two patients with acute lymphoblastic leukaemia and complete metabolic remission in a third with diffuse large B-cell lymphoma (DLBCL). CAR-T cell generated using the PiggyBac transposon system with IL-15 and co-cultured with peripheral-blood mononuclear cells showed between 5-10% of TSM-cells. CAR-T cells expanded in blood, produced cytokine-mediated toxins commensurate to tumour-burden, and had up to 200 CAR-T/μl blood at 3-months follow-up. scRNAseq from the total CD3+ or CD8+ (CD45RA-CD95+CXCR3+) compartments had a median of 858-950 genes per cell, with multiple gene-clusters identified. TSM showed 4-fold enriched by cell cycle with proliferation capacity and fatty-acid oxidation, in line with recent findings on O-IIBB CAR-T compared to CD28CAR-TCI. Full-length TCR was identified in 20% of the cells, and at least one chain in 75% of the cells. Ongoing research on post-infusion blood samples seeks identification of TSM clones that are maintained in the blood. This study reveals heterogeneous populations of cells in the CAR T product, which can confer long-term survival of cells in the patient and minimise toxicity.

P.E3E4.01.08
TCR sequence motif based classification of CD4-CD8 cells

E. Ofitserov1, V. Tsvetkov1, V. I. Nazarov2; 1Tula State University, Tula, Russian Federation; 2Pirogov Russian National Research Medical University, Moscow, Russian Federation.

The field of immunology has witnessed an exponential growth of data. For instance, accumulation of TCR sequencing data allowed classification of CMV status in patients. This is the very point where big data algorithms should step in. Nevertheless, conventional deep neural networks (NN) lack of interpretability due to poor algorithmic transparency. In order to combine interpretability and performance we sought to develop a comprehensive NN training algorithm for motif-based sequence classification. It incorporates differential solving and refinement that provides end-to-end learning without preprocessing. Short substrings with gaps - TCR motifs - are generated during model training that constitute the features for the classification. In other words generated sequences are the cell type associated motifs as well as a part of NN classification model. The method was applied to human TCR sequencing data to classify CD4 and CD8 cell subsets. This analysis revealed the CD4 and CD8 associated motifs with the mean accuracy of prediction at least 70% across all repertoires. Thus it is an intelligible way to discern the measurable difference between cell subsets.

P.E3E4.01.09
HLA Frequencies in a population of Barranquilla, Colombia

C. H. Parga Lozano1, M. De Bourayne2, D. Berdnik3, E. Z. TASKIRAN3; 1Universidad Libre, Barranquilla, Colombia; 2Universidad de los Andes, Bogotá, Colombia; 3Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, United States.

Introduction: Barranquilla is a city in northern Colombia. The city has high immigration in the last 200 years, mainly from Central Asia, Africa and Europe. That caused a significant miscegenation of the population along with native Amerindians in the region. Materials and methods: 43 individuals from Barranquilla were typed for the detection of the HLA-DRB1 and DQB1 alleles. The HLA allele frequencies of 738 chromosomes were analyzed and compared these with 9 Colombian Amerindian populations using genetic data analysis. The most frequent alleles were DRB1*15:01(0.08889), DQB1*01:01(0.077778), DRB1*04:01(0.077778) and DQB1*04:01(0.077778). DRB1*04:01(0.077778) and DQB1*04:01(0.077778) showed association with the pre-stimulation and day 10 post-stimulation repertoire. Methods: We analyzed sequential samples - taken in triplicate - during 10 day peptide-based in vitro T-cell stimulation assays using NGS-based T-cell receptor (TCR) repertoire analysis (NGS-TCR-RA) to identify, fingerprint and monitor antigen-specific T-cell responses at the clonal level. Results: Reproducible gradual expansion of initially low-frequency T-cell clones was observed during the cultures. We developed a statistical approach to identify differentially expanded, candidate antigen-specific TCR clones by comparing the pre-stimulation and post day 10 post-stimulation repertoire. We confirmed antigen-specificity of most significantly expanding clones using NGS-TCR-RA of T-cells sorted on CD40L expression upon short antigen re-challenge. Conclusion: NGS-TCR-RA is a robust, sensitive and reproducible tool for identification and monitoring of antigen-specific T-cell responses, performing statistical evaluation at the single clone level. Moreover, based on clonal fingerprinting it is able to link in vitro analysis of clones to in vivo observations on T-cell responses. As such it constitutes a novel, powerful “omics” tool to fingerprint relevant B- and T-cells in adaptive immune responses.

P.E3E4.01.10
A novel statistical approach to monitor clonal antigen-specific T-cell responses using targeted RNA-seq

S. Pollostra1, M. De Bouranye2, B. van Schaik3, B. van Kampen1, A. van Kampen2, B. Mailletere4, N. de Vries5; 1AMC, Amsterdam, Netherlands, 2SimPRO, CEA, Saclay, France.

Introduction: In vitro T-cell stimulation assays are basic immunological tools used to investigate antigen-specific T cell responses. Here we explored different experimental set-ups to take this tool to the single clone level using next generation sequencing (NGS) based technologies and novel statistical approaches. Methods: We analyzed sequential samples - taken in triplicate - during 10 day peptide-based in vitro T-cell stimulation assays using NGS-based T-cell receptor (TCR) repertoire analysis (NGS-TCR-RA) to identify, fingerprint and monitor antigen-specific T-cell responses at the clonal level. Results: Reproducible gradual expansion of initially low-frequency T-cell clones were observed during the cultures. We developed a statistical approach to identify differentially expanded, candidate antigen-specific TCR clones by comparing the pre-stimulation and day 10 post-stimulation repertoire. We confirmed antigen-specificity of most significantly expanding clones using NGS-TCR-RA of T-cells sorted on CD40L expression upon short antigen re-challenge. Conclusion: NGS-TCR-RA is a robust, sensitive and reproducible tool for identification and monitoring of antigen-specific T-cell responses, performing statistical evaluation at the single clone level. Moreover, based on clonal fingerprinting it is able to link in vitro analysis of clones to in vivo observations on T-cell responses. As such it constitutes a novel, powerful “omics” tool to fingerprint relevant B- and T-cells in adaptive immune responses.

P.E3E4.01.11
Communomice analyses identify the TNF/TRAIRf receptor family as a potential determinant of virus control and CD4 T cells counts in chronic natural HIV infection

M. Ruiz-Riof1, B. Orio-Tordera1, A. Llano1, D. Berdnik1, E. Z. TASKIRAN2, V. Tsvetkov1, B. Oriol-Tordera1, G. Gómez1, C. Ganoza2, T. Wyss-Coray3, 1IrissiCaixa, AIDS Research Institute, Badalona, Spain, 2Universitat de Vic - Universitat Central de Catalunya (UdV-UCC), Vic, Spain, 3Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, United States, 4Instituto de Investigaciones de la Ciencia y la Evolución (ICNEA), Barcelona, Spain, 5IMPACTA, Lima, Peru, 6Universitat Politècnica de Catalunya-Barcelona Tech, Barcelona, Spain.

The progressive loss of CD4 T-cells in untreated HIV infection is incompletely understood. Apart from direct cytolytic effects of viral replication, “bystander” apoptosis triggered by soluble factors or membrane-bound host immune factors also contribute to cell death of infected and uninfected cells. To identify soluble plasma factors driving cell-death during chronic HIV infection, “communomice” profiles were assessed in HIV-infected, treatment-naive individuals with high (>50,000, n=47) or low (<10,000 HIV RNA copies/ml, n=49) viral loads. Multivariate classification and regression model analyses for CD4 count prediction revealed that plasma levels of soluble death factors, most of them involved in TNF (tumor necrosis factor) receptor dependent apoptosis-inducing ligand signaling, are critical for control of viral replication and CD4 loss.
In particular, plasma concentrations of two TRAIL decay receptors (TRAILR3 and TRAILR4) were significantly correlated with peripheral blood CD4 T cell counts as well as viral load and CD4:CD8 ratios. A genome-wide analysis of basal gene expression levels supported these associations further. An additional TNF ligand (TLLA; TNFSF15) and TNF receptor (DR3; TNFRSF25), were strongly related with CD4 counts. These results were validated in unrelated cohorts of seronegatives, recently HIV infected subjects (3-6 months post-seroconversion), longitudinally followed individuals tested 1 year before and after cART initiation and HIV controllers. The data identify several soluble plasma markers of the TNFR family that are related to HIV disease progression and which are involved in “bystander” cell death, thereby opening potential new targets for immune-based therapeutic interventions in HIV infection.

P.E3E4.01.12
A mathematical model of TCR signaling and its information transmission in a pool of low affinity ligands

K. Seok, R. I. de Boer; Utrecht University, Utrecht, Netherlands.

T cells scan the surface of antigen presenting cells to detect foreign peptides on major histocompatibility complex (MHC). Recognition takes place via the binding of T cell receptor (TCR) to peptide-MHC and the transduction of downstream signaling cascades. To induce a proper immune response, it is essential that T cells are activated when they bind to foreign peptides and not when confronted with self-peptides. Although T cells that have high affinity to self-peptides are deleted during development in the thymus, T cells in the periphery should still discriminate foreign peptides with high affinity to its TCR from the many and abundant low affinity self peptides. Failures in this discrimination result in either infection or autoimmune diseases. Many models have been suggested to explain this, however, it is not clear that how peptide discrimination by TCRs is disturbed in the background of many low affinity self peptides that could antagonize signaling. Here we simulate the TCR signaling model developed by Francois et al. 2013 PNAS with low affinity ligands, and quantify the ability of ligand discrimination using mutual information. The mutual information indicates the maximum number of input signal values that a signaling pathway can reliably resolve. The results show that ligand discrimination works only in the presence of antagonists with very low affinity, which suggests that negative selection of immature T cells should be very strict. We will also discuss how the simulation of immature T cells with different strength of TCR signaling results in different T cell differentiation.

P.E3E4.01.13
Development of recombinant antibodies: highly reproducible with tailored specificity

M. Sassi, A. Wittmann, A. Symonds, R. Adams, A. Solache, B. Hamilton; Abcam plc, Cambridge, United Kingdom.

Recombinant Antibodies are fundamental tools in both basic and clinical research of immunological targets. However, an increasing number of studies have shown that not all antibodies are specific, which leads to a lack of experimental reproducibility. To provide antibodies that have excellent specificity and reproducibility, we have engineered recombinant versions of our RabMab® rabbit monoclonal antibodies. Recombinant antibodies are manufactured by cloning the immune specific heavy and light antibody chains into a high-yield mammalian expression vector or they can be produced from an existing hybridoma. The technology combines the superior antigen recognition of a rabbit immune system with the specificity and consistency of a monoclonal antibody. Our RabMab® products are validated in key applications (western blot, IHC, ICC/IF, IP, and flow cytometry) and include several relevant targets in immunology and immuno-oncology research. Successfully validated products will be produced in various formats (Fluorescent conjugates, PBS formulations) to allow their use in many types of in vivo and in vitro studies. Our phage display technology is an alternative method to generate high-affinity binders against difficult proteins, small molecules, and toxins. This in vitro approach is based upon a large library of bacteriophage particles, each carrying the genetic information and the unique phenootypic binding function of one antibody clone. Utilizing both in vivo and in vitro technologies, we can deliver high quality antibodies to all targets. The advantages of recombinant antibodies over both traditional monoclonal and polyclonal antibodies are increased in consistency and reproducibility, high affinity and specificity, ease of scalability.

P.E3E4.01.14
Quantifying immune cell subsets in living cultures over time using IncuCyte® live-cell analysis


CD surface markers have long been used to identify immune cell subsets and associate cells with certain immune functions. Typically, flow cytometry and specific fluorescently-labeled antibodies are used for these analyses. Whilst extremely powerful, this approach does not readily afford in real-time into temporal changes or select interactions between cell populations in heterogeneous systems. Here we describe a novel labelling and analysis strategy to enable long-term, non-invasive quantification of immune cells based on IncuCyte® live-cell imaging.

An Fc-targeted anti-mouse Fab fragment conjugated to a green-emitting fluorochrome (Incucyte F(ab)Fluor-488) was used to tag antibodies to cell surface markers. Addition of the F(ab)Fluor-488 antibody complex to living cells, including OptiGreen background suppressor, produced fluorescent labelling that was sufficiently bright and stable to allow repeated measurements for 4 days without perturbing cell morphology or growth. With new image analysis and visualisation tools, individual cells were segmented from the phase-contrast image and quantified cell by cell for fluorescence. The method was validated by comparing the phase cell counts over time with nuclear fluorescence values in proliferating Jurkat-NKxLight RFP cells. In PBMCs, the anticipated frequencies of lymphocyte subpopulations (CD4, CD8, CD45) were detected using this method. Cell subsets can be gated for further analyses, including dynamic changes in response to stimuli, e.g. increased immune responses. Using the Image Analysis plug-in, our antibodies can be used for quantification of immune cell subsets in cultures over time. This method should prove powerful in analyses of dynamic heterogeneous cell models and for studying cell-cell interactions.

P.E3E4.01.15
Define the dynamic protein landscape during early of human Th17 cell differentiation


Th17 cells play key role in the pathogenesis of autoimmune and immune diseases and in various cancer. Hence, it is critical to unveil molecular signatures driving the differentiation of Th17 cells in order to understand their regulation during diseased state. We performed label-free mass spectrometry based quantitative proteomics analyses to reveal the Th17 cell-specific proteomic signature regulating Th17 cell differentiation and function in human. We compared the human Th17 proteomics data generated in this study with our previously published data on the transcriptomic profiles during human Th17 differentiation that revealed the degree of similarities and differences between the transcript and the protein levels. Furthermore, we validated a panel of selected proteins with known and unknown functions in Th17 cell differentiation. To our knowledge, this study is the first to map the global protein landscape during early human Th17 cell differentiation.

P.E3E4.01.16
Inflamed tissue factors contribute to the emergence of auto-reactivity in granulomatosis with polyangiitis

G. Weppner, O. Oehler, C. Hammers, K. Holl-Ulrich, K. Hessebacher, G. Riemelkasten, S. Ibrahim, A. Recke, P. Lamping, A. Muller; 1Department of Rheumatology & Clinical Immunology, Luebeck, Germany, 2IGA, Luebeck, Germany, 3Department of Dermatology, Luebeck, Germany, 4Institute of Pathology, Marienkrankenhaus, Hamburg, Germany, 5Department of Otorhinolaryngology, Luebeck, Germany.

Circulating anti-neutrophilic cytoplasmic autoantibodies targeting proteinase 3 (PR3-ANCA) are a diagnostic and pathogenic hallmark of granulomatosis with polyangiitis (GPA). It is, however, incompletely understood if immune tissue support presence and emergence of PR3-ANCA. Using immunofluorescence staining for IgG and a common PR3-ANCA antibody (5/7)1 was undertaken. To gain insight into surrogate markers possibly indicative of a P3R-driven antibody response at inflamed sites, a meta-analysis comprising IgGV and IgGV originating from respiratory tract tissue of GPA (231 clones) was performed. Next generation sequencing-based IgGV genes derived from peripheral blood of healthy donors (244,353 clones) and previously published IgGV genes (148 clones) served as controls. For comparison, Ig genes of murine and human monoclonal anti-PR3 antibodies were analyzed. Few 5/7 Id IgG B-cells were detected in inflamed tissue of GPA. IgGV and IgGV derived from inflamed tissue of GPA displayed altered V(D)J usage, contributing to prolonged complementarity determining region 3 (CDR3) in the IgGV genes. Further, selection against amino acid exchanges was prominent in the framework region of IgGV genes derived from inflamed tissue of GPA. Comparing V(D)J rearrangements and deduced amino acid sequences of the CDR3 between anti-PR3 antibodies and Ig clones derived from inflamed tissue of GPA, yielded no identities and few similarities. Thus, few PR3-ANCA B-cells were found in inflamed tissue of GPA. For the first time, the search for clones producing PR3-ANCA IgG in inflamed tissue might require methods that can detect rare clones.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 521

POSTER PRESENTATIONS
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands.
**POSTER PRESENTATIONS**

**P.E4.01.02**

**Binding between recombinant Phospholipases D from the venom of Loxosceles laeta and “Lipid Rafts” from the membrane of human monocyte THP-1 activates the PI3k /Akt pathway.**

T. A. Arnd-Scel, J. M. Rojas, I. E. Araya, A. Cataldo; Universidad de Antofagasta, Antofagasta, Chile.

**Introduction:** Lipid Rafts are dynamic complexes, located on the cell surface, mainly composed of cholesterol, sphingolipids and proteins. It’s believed that its most important role is to participate in the transduction of signals into the cell. Studies in mouse T cells have shown an association between the Lipid Rafts and the PI3k /Akt pathway, facilitating membrane recruitment, and activation of Akt. **Aim:** Demonstrate that the association between recombinant Phospholipases D of the venom Loxosceles laeta and Lipid Rafts of the monocyte THP-1 membrane is able to activate the PI3k /Akt pathway. **Methodology:** Using recombinant isoforms of the Loxosceles laeta venom, the interaction between our proteins with Lipid Rafts of the monocyte THP-1 membrane was evaluated by immunofluorescence. Also, activation of the PI3k/Akt pathway was evaluated by Western Blot of proteins from THP-1 cells incubated with our recombinant proteins. **Results:** From the incubation with antibodies specific for Lipids Rafts, recombinant Phospholipases, marked with fluorophores, it was possible to demonstrate co-localization between our recombinant proteins and the Lipid rafts from THP-1 cells. Also, this cell line, when incubated with recombinant Phospholipases D, showed changes in the PI3k/Akt pathway evaluated by Western Blot. **Conclusion:** No previous report has addressed the possible association of Lipid Rafts to the signals induced by Phospholipases D of Loxosceles spiders. In addition, its role in the activation of the PI3k/Akt signaling pathway related to the production of cytokines and chemokines associated with the inflammatory response to Loxosceles venom its unknown. So, according to the results, our work contributes to the understanding of this problem.

**P.E4.01.03**

**Polymorphism analysis of TLR7 (rs179008) and TLR9 (rs352140) genes in systemic lupus erythematous patients**

M. A. Bashir, N. Afzal, R. Tahir, F. Shahzad, M. Kashif, S. Jahan; University of Health Sciences, Lahore, Pakistan.

**Background:** Systemic Lupus Erythematosus (SLE) is an inflammatory autoimmune disease characterized by production of autoantibodies and subsequent damage to multiple organs. In SLE, various antibodies are formed and mostly anti-dsDNA levels are raised in serum of SLE patients. Various genome wide studies have shown association of TLR7 and TLR9 genes with SLE. Therefore, this study was designed to determine and compare single nucleotide polymorphism (SNP) at restriction sites of TLR7 gene (rs179008) and TLR9 gene (rs352140) between local population of SLE patients (Group-1) and healthy controls (Group-2). **Method:** It was a case control study. Eighty samples were recruited for each of the two study groups. Three ml of EDTA blood from patients and control was collected and processed for the analysis of gene polymorphism of TLR7 (rs179008) and TLR9 (rs352140) by PCR-RFLP after DNA extraction. Chi-square test was used to analyze polymorphism analysis and allele frequencies between two groups. Associations of TLR 7 and TLR 9 gene polymorphism with SLE and its clinical parameter were analyzed. **Results:** In TLR7 genotype AT and TT are not significantly associated with SLE while TLR9 CT genotype and TT genotype and especially T allele are significantly associated with SLE showing significant interdependence of TLR9 gene polymorphism with SLE patients. **Conclusion:** Genetic variation in TLR9 may be a key part in pathogenesis of SLE; therefore and T allele and TT genotype is associated with SLE so further studies are needed to establish this genetic factor as biomarker for local population.

**P.E4.01.04**

**N-3PUFAs reduce CD4+ T-cell distribution to adipose tissue via both cellular & systemic effects**

D. Cucchi, M. D. Camacho-Munoz, J. Smith, A. Nicolau, C. Maurer1; 1University of Warwick Research Institute, London, United Kingdom, 2Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom, 3The Institute of Cancer Research, London, United Kingdom, 4Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom.

**Background:** Lipid imbalance observed in cardiovascular metabolic disorders (CVMD) alters T-cell membrane lipid composition, mediator production and related signalling cascades, leading to unwanted inflammatory responses. We postulated that these alterations may be corrected by omega-3-polyunsaturated fatty acids (n-3PUFAs). Indeed we showed that exposure of activated T-cells to bioactive lipids can modify migratory patterns in vitro and in vivo and we investigate their mechanisms of actions. **Materials and Methods:** In mice fed n-3PUFA-enriched diet for 3 weeks, we found a significant reduction in pro-inflammatory CD4+ T-cells in adipose tissue, as well as significant changes in the lipids profile in plasma and adipose tissues, consistent with an anti-inflammatory signature. **Results:** We better understand the effect of n-3PUFA on T-cell motility, we assessed CD4+ T-cell migratory capabilities in vitro upon treatment with EPA and DHA in a trans-endothelial migration assay, observing a significant reduction of migration. Furthermore, when T-cells were pre-treated with EPA and DHA in vitro and then implanted in mice, they migrated to the inflamed peritoneum at much lower numbers. In line with this, we found that EPA and DHA treatments are able to reduce the number of polarised T-cells in vitro, alter membrane microdomains and decrease the activity of small Rho GTPases, whose role in cytoskeletal dynamics is crucial. **Conclusions:** These findings show that the two principle n-3PUFA, EPA and DHA are active in reducing the motility of CD4+ T-cells and their ability to reach target tissues through changes in the cytoskeleton.

**P.E4.01.05**

**Exploring mir-34c-5p modulation of effector CD4+ T-cell differentiation through logical modelling**

N. Domingues, F. C. Pinto, A. E. Sousa, M. Gama-Carvalho; 1Biostl - Biosistemas e integrative sciences institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal, 2Molecular Medicine Institute, Faculty of Medicine, University of Lisbon, Lisbon, Portugal.

**Background:** miRNAs are essential for proper immune cell development and function. mir-34c-5p is upregulated in naïve CD4 T cells 72 hours after in vitro stimulation, but its role in activated T cells remains unknown. The already complex signalling aspects of the immune system are exacerbated by miRNA regulation. Modelling approaches can be extremely useful for understanding the regulation of miRNAs are essential for proper immune cell development and function. **Materials and Methods:** It was a case control study. Eighty samples were recruited for each of the two study groups. Three ml of EDTA blood from patients and control was collected and processed for the analysis of gene polymorphism of TLR7 (rs179008) and TLR9 (rs352140) by PCR-RFLP after DNA extraction. Chi-square test was used to analyze polymorphism analysis and allele frequencies between two groups. Associations of TLR 7 and TLR 9 gene polymorphism with SLE and its clinical parameter were analyzed. **Results:** In TLR7 genotype AT and TT are not significantly associated with SLE while TLR9 CT genotype and TT genotype and especially T allele are significantly associated with SLE showing significant interdependence of TLR9 gene polymorphism with SLE patients. **Conclusion:** Genetic variation in TLR9 may be a key part in pathogenesis of SLE; therefore and T allele and TT genotype is associated with SLE so further studies are needed to establish this genetic factor as biomarker for local population.

**P.E4.01.06**

**The role of integrins in serine protease activated protein C signalling in T-cell**

D. Gupta, S. Ranjan1, S. Kohli1, R. Rana, A. Muller, B. Schraven, B. Isemann; 1Department of Clinical Chemistry and Pathobiology, Magdeburg, Germany.

**Introduction:** The serine protease activated protein C (aPC) is an anticoagulant, which conveys intracellular signals via its interaction with integrins to mediate its protective effect in GvHD. **Methodology:** Using recombinant isoforms of the aPC in anti-CD3 and anti-CD28 stimulated naive CD4 T cells in the presence of IL2, using an asynchronous updating scheme. Using a specific set of logical rules, we identified GATA3, FOS and MYC, in addition to other factors in in vitro and in vivo. **Results:** We found a significant reduction in pro-inflammatory CD4+ T-cells in adipose tissue, as well as significant changes in the lipids profile in plasma and adipose tissues, consistent with an anti-inflammatory signature. **Conclusion:** We better understand the effect of n-3PUFA on T-cell motility, we assessed CD4+ T-cell migratory capabilities in vitro upon treatment with EPA and DHA in a trans-endothelial migration assay, observing a significant reduction of migration. Furthermore, when T-cells were pre-treated with EPA and DHA in vitro and then implanted in mice, they migrated to the inflamed peritoneum at much lower numbers. In line with this, we found that EPA and DHA treatments are able to reduce the number of polarised T-cells in vitro, alter membrane microdomains and decrease the activity of small Rho GTPases, whose role in cytoskeletal dynamics is crucial. **Conclusions:** These findings show that the two principle n-3PUFA, EPA and DHA are active in reducing the motility of CD4+ T-cells and their ability to reach target tissues through changes in the cytoskeleton.
POSTER PRESENTATIONS

P.E4.01.04
The role of ROS hyperproduction in murine model of autoinflammatory osteomyelitis

J. Kralove1, A. Drobeli1, J. Prochazka1, S. Poupout2, D. Glazov3, S. Borna1, P. Angelovski1, T. Skocpava1, J. Pokorna1, R. Sedlacek4,1, T. Brdiczka1.
1Institute of Molecular Genetics of the ASCR, Prague, Czech Republic; 2Institute of Hematology and Blood Transfusion, Prague, Czech Republic; 3Institute of Endocrinology ASCR, Prague, Czech Republic; 4University of Veterinary Sciences, Prague, Czech Republic.

PSTP12 is an adaptor protein expressed in the myeloid cells and its deficiency leads to the development of auto-inflammatory disease in mice. This disease has been designated chronic multifocal osteomyelitis (CMO) as the main manifestation is sterile inflammation of the bones accompanied by inflammation of the soft tissues in the external parts of the bone. We found production of pro-inflammatory cytokine IL-1β by murine neutrophils is the main factor driving CMO development and progression. In addition, high-activation of various signaling pathways in CMO neutrophils has been observed. The negative regulatory effect of PSTP12 protein on signaling pathways and IL-1β production is likely mediated by its interacting partners. These include inhibitory molecules, such as CSK, SHIP1 and protein tyrosine phosphatases from the proline-, glutamic acid-, serine- and threonine-rich (PEST) family. However, the exact mechanism of how PSTP12 inhibits signaling is not known. We here describe deregulated ROS production in CMO bone marrow cells and purified neutrophils. We investigate the role of ROS in CMO disease development and progression, CMO mice with non-functional protein (PSTP12 knockout; Novozymes) were generated. Detailed analysis of this mouse strain including disease free curves, CT scans and IL-1β production was performed and data will be presented showing partial alleviation of the disease symptoms.

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P.E4.01.09
RNAseq for novel sialic acid inhibiting signalling via Siglec in moDCs

1Clinica Medica Central, Amsterdam, Netherlands; 2UvA, Amsterdam, Netherlands.

Cancer cells and pathogens, use sialic acids to actively inhibit the immune system by binding Sialic-acid binding immunoglobulin type lectins (Siglecs) with an ITIM motif. This motif becomes phosphorylated; SHP binds and phosphorylates downstream targets, triggering inhibitory processes. In mice bone marrow-derived DC, we have shown that sialic acid modified antigens are internalized and are used as a regulatory program on DCs, inducing Tregs and inhibiting generation of Teffector cells. To study whether similar inhibitory processes are induced by sialic acids on human monocyte-derived DCs (moDCs), displaying Siglec, 1, 7, and 9, we generated a2,3 and a2,6 sialic acids on dendrimers (a2,6-dendrimers). MoDCs binding of these dendrimers enhanced IL-10 and reduced IL-12p70 production, pointing towards a more tolerogenic phenotype. To explore the pathways induced by these sialic acids, we performed an unbiased screen of RNAseq data from moDCs treated with a2,3 and a2,6 siala-dendrimers.

a2,3 si-dendrimer binding to moDCs uncovered 867 uniquely differentially expressed genes (DEGs) and a2,6-si-dendrimer revealed 47 DEGs. Gene Set Enrichment Analysis (GSEA) of a2,3 si-dendrimer enriched in pathways regulating many pathways including in the high pathway enrichment significance. The sub-pathways cytokine signaling and antigen processing and proteasome degradation were enriched, especially the ubiquitination sub-pathway were most pathway hubs were changed. In the a2,6-si-dendrimer only half of the hubs in this sub-pathway were changed. This indicates that siylated dendrimers are processed differently and thereby influence antigen processing and presentation leading to changes in T cell polarisation.

P.E4.01.10
Granzyme B in the cell line NK-92: novel processes and old routines

A. V. Korenevsky, Y. P. Miljutina, A. A. Zhdanova, A. D. Scherbitskaya, V. A. Semenov, V. A. Mikhailov, D. I. Sokolov, S. A. Selkov;

Introduction: At present, a variety of routine methods is used for the proteome evaluation. In this study, we have focused on the search for granzyme B in the cell line NK-92 using combination of novel approaches. Material and Methods: The cell line NK-92; 2D-electrophoresis (Protean i12 IEF Cell, PowerPac HC, BioRad, USA), isoelectric focusing (3100 OFFGEL fractionator); Agilent Technologies, USA, on-chip electrophoresis (bioanalyzer 2100, Agilent Technologies, USA), Western blotting. Results: The routine 2D-electrophoresis followed by MS/MS identification was accompanied by a long search for granzyme B and was no success, as staining with Coomassie had not visualized the relevant spot. Therefore, the proteins were subjected to 1D-electrophoresis followed by Western blotting to discover the target protein. The novel approach was that the cell lysate was divided into 24 soluble fractions depending on isoelectric point, which allowed for 1D-electrophoresis followed by Western blotting and subsequent identification of the fraction containing the highest amount of granzyme B. Additionally, the protein profiles of the obtained fractions were then compared using on-chip electrophoresis. The advantage of this approach is significant timesaving, when compared to the Laemmli method. Besides, the remaining fractions allows performing standard mass-spectrometry or separating proteins using micropreparative HPLC with subsequent MS/MS identification. Conclusions: The combination of novel approaches using routine methods allow for more valid granzyme B identification in the cell line NK-92. Supported by RFBR grant #17-04-00679 and President’s grant NSh-2873.2018.7. The study was performed using equipment of the SPbSU Science Park resource center “Development of molecular and cellular technologies.”

P.E4.01.11
Mesenchymal stem cells from healthy human gingiva produce lower levels of IL-6 compared to their counterpart from chronic periododontitis

M. Milinković1, M. Marković1, I. Majstorović1, M. Milanović1, S. Želević1, S. Tadorović1, M. Cilić1;
1University of East Sarajevo, Medical Faculty R, Srpiska, BH, Foca, Bosnia and Herzegovina; 2University of Defence in Belgrade, Medical Faculty of the Military Medical Academy, Belgrade, Serbia; 3University of Defence in Belgrade, Medical Faculty R, Srpiska, BH, University of Defence in Belgrade, Medical Faculty of the Military Medical Academy, Belgrade, Serbia, University of East Sarajevo, Medical Faculty R, Srpiska, BH, University of Defence in Belgrade, Institute for Application of Nuclear Energy, Belgrade, Serbia, Belgrade, Serbia.

Mesenchymal stem cells (MSCs) are isolated and characterized from different dental tissues, including gingiva. However, little is know whether and how chronic inflammation changes their functions. The aim of this study was to compare phenotypic profile, differentiation potential and cytokine production between MSCs isolated from human healthy gingiva and gingiva from chronic periodontitis patients. We showed that 90-98% of both types of MSCs expressed typical markers such as CD90, CD73 and CD105 and were able to differentiate into osteoblasts, chondroblasts and adipocytes under appropriate cell culture conditions. The expression of other markers, including CD146, CD56, STRA-1 and PDGF-R was lower on MSCs from healthy gingiva, but their proportion in both groups was variable, depending on the donor and number of culture passages. By using fluorescent and confocal microscopy, we demonstrated that pericytes, supposed to be the dominant source of these MSCs in vivo, expressed strongly NG2, PDGFR, CD146, CD105 and CD106, but not CD34 and CD31, the markers of endothelial cells. The relative number of pericytes was higher in diseased gingiva. Both types of MSCs produced IL-6, but its level was significantly lower in MSCs from functional gingiva as compared with periodontal tissue explant of gingival biopsies from chronic periododontitis patients, through enhanced production of IL-6 by MSCs from healthy gingiva, suggesting that gingival MSCs during periodontitis, through enhanced production of IL-6, could have a proinflammatory role.

P.E4.01.12
Immunophenotyping extracellular vesicles using Amnis imaging technology

H. R. Pugsley, B. R. Davidson, P. Morrissey;
Mercck, Seattle, United States.

Only recently has the importance of extracellular vesicles (EVs) as key mediators of intercellular communication been appreciated. EVs are membrane derived structures that include exosomes, microvesicles and apoptotic bodies. Exosomes have been shown to transfer molecules between cells and have the potential to transfer signals between cells. Exosomes play an important role in normal physiology and cancer, and it is now known that they can also serve as mediators in the pathogenesis of neurological, vascular, hematological and autoimmune diseases as well as cancer. Quantifying and characterizing EVs in a reproducible and reliable manner has been difficult due to their small size (exosomes range from 30 - 100 nm in diameter). EV analysis can be performed using high-magnification microscopy however this technique has a very low throughput. Attempts to analyze EVs using traditional PMT based flow cytometers has been hampered by the limit of detection of such small particles and low refractive index. To overcome these limitations we have employed the recently developed CellStream flow cytometer. The CellStream utilizes the Amnis imaging technology, having the advantage of high throughput flow cytometry with higher sensitivity to small particles due to the time-delay-integration image capturing system. In this study, the CellStream was used to immunophenotype EVs derived from red blood cells and platelets.

P.E4.01.13
Identifying exosome binding and internalization in blood cell subsets by multispectral imaging flow cytometry

H. R. Pugsley, S. L. Friend, B. E. Hall, P. Morrissey;
Mercck, Seattle, United States.

Exosomes have been shown to transfer molecules between cells and have the potential to transfer signals between cells. To study how exosomes are interacting with white blood cells high-magnification microscopy can be used; however, this technique has very low throughput. In addition, these events are rare and therefore difficult to analyze objectively and statistically by traditional microscopy methods. To overcome these limitations we have employed multispectral imaging flow cytometry that has the advantage of combining high throughput flow cytometry with high sensitivity fluorescence microscopy. In this study we use multispectral imaging flow cytometry to investigate the interaction of exosomes with white blood cells. Exosomes derived from Jurkat cells were labeled with anti-human CD263-AF647 and added to human white blood cells.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

524
The cells were labeled for immunophenotyping, fixed, and then labeled with anti-human CD63-PE to identify external exosomes. Phospholipase D (PLD) family enzymes from Loxosceles spider venom, are responsible of dermonecrosis during human spider envenomation. On human skin fibroblasts, different recombinant isoforms of PLDs from L. laeta, induced IL-6, IL-8, CXCL1, and CCL2/MCP-1 production. However, the signaling pathway implicated in this process is unknown. Aim: Evaluate the signaling pathway involved in chemokines production mediated by Loxosceles PLDs on skin fibroblast. Methodology: Human skin fibroblasts HFF-1 cultures were incubated with recombinant PLDs from L. laeta (rPLD1 and rPLD2), and the mutant isoforms of rPLD1 (rPLD1-D259G and rPLD1-D256S), and the Akt activation were evaluated by western blot, using an anti-phospho-Akt/PI3Kα (Ser473) monoclonal antibody. Additionally, PI3K participation was determined by Western Blot of phosphorylated Akt in presence or absence of PLDs plus wortmannin. The PLDs-lipid raft binding was evaluated by immunofluorescence using kit Vybrant® Lipid Raft labeling kit

1 cultures were incubated with recombinant PLDs from University Hospital of Bern, Bern, Germany.

Upon activation T lymphocytes rapidly aggregate and form cell communities both in vitro and in vivo in an LFA1-ICAM1 dependent manner. The close proximity of cells within clusters promoting internalization of cell surface transmembrane messengers and/or direct cell interaction. We hypothesized that within clusters activated T cells mutually regulate their behaviors and subsequent differentiation akin to quorum-regulation of bacteria. In order to identify molecules involved in such crosstalk, we have developed a bioinformatics-framework allowing us to identify receptor-ligand pair co-expressed on activated T cells. We then characterized selected interactions and revealed that within clusters cellular crosstalk sustains T cell expansion by limiting activation-induced cell death. Furthermore, we found evidence for an additional related counter-regulatory signaling circuit which limits further T cell expansion. Competiton between these two signaling circuits shapes the outcome of T cell activation. In summary, our results indicate that T cell clusters serve as hubs for mutual regulation of activated T cells. Such behavioral coordination within a defined spatiotemporal framework enables the generation of robust yet flexible immune responses at the population level.

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P.E4.01.14 Establishment of an engineered antigen presenting cell platform to study antigen specific human CDB T cell responses in vitro

M. Reithofer1, S. Roskopf2, C. Battini3, J. Leitner3, B. Bohle1, B. John-Schmid1, P. Steinberger1

1Institut für Pathophysiologie and Alleryresearch, MCCA PhD Programme, Medical University, Vienna, Austria, 2Institute of Immunology, Medical University, Vienna, Austria, 3Institute for Pathophysiology and Allergyresearch, Medical University, Vienna, Austria.

Studies on antigen-specific human CDB T cells are hampered by their low frequency in the peripheral blood. Expansion of these cells in vitro by antigenic peptide is inefficient and often precludes a thorough characterization. Here we describe a cellular platform which allows to efficiently stimulate and expand antigen-specific human CDB T cells. HLA-A201, the most common MHC class I molecule in the Western population was expressed on human K562 cells. In addition, we introduced an artificial gene encoding five major HLA-A2-restricted viral epitopes targeted into the proteosomal degradation and antigen processing pathway. Upon coculture with PBMCs of HLA-A2 positive human donors, the eAPC efficiently expanded virus-specific T cells. Additionally generated eAPC expressing costimulatory ligands like CD80, CD86 or 4-1BBL had augmented capacity to stimulate antigen-specific T cells. Cytotoxicity assays confirmed that stimulation with eAPC yielded potent CDB effector T cells. MHC class I tetramer staining experiments were performed to identify stimulation conditions that promoted the expansion of antigen-specific T cells and prevented bystander T cell activation. Taken together, our results indicate that we successfully established an eAPC platform for T cells to emulate and facilitate the function of accessory molecules in the expansion of antigen-specific human CDB T cells. This system may be applied for the development of improved protocols for in vitro generation of virus and tumour-specific T cells for adoptive therapy.

P.E4.01.15 Analysis of miRNA involvement in CD4+T cell differentiation

G. A. Rockinger1, P. Röell1, D. Zehn1, G. Scholz1, P. Romero1, C. Jandus2

1University of Lausanne, Epalinges, Switzerland, 2Technical University of Munich, Munich, Germany.

Introduction: in contrast to CD8+ T cell, CD4+ T cell have only recently gained increasing importance in tumor immunity as studies showed their therapeutic relevance, including the regulation of adaptive immunity. A key to CD4+ T cell immunotherapy will depend on a better understanding of the regulation of CD4+ T cell differentiation, to promote stem cell memory (SCM) and central memory (CM) phenotypes.

Material and methods: we performed a mRNA sequencing and a microRNA (miR) array of highly-purified sorted naïve (N), SCM, CM and effector memory (EM) CD4+ T cell subsets from peripheral blood of 4 healthy donors, followed by in depth Bioinformatic analysis and in vitro target validation.

Results: we identified different expression between N, SCM, CM and EM cells of known miR such as miR-144-5p and miR-155-5p and of previously underscribed miR. Further investigations in additional healthy donors’ samples confirmed by qPCR the differential expression of these miR. Further, we were able to correlate the expression of candidate miR that with up or downregulation of targeted genes with CD4+ T cell subset of interest.

Conclusion: we are presently investigating miR and target mRNA expression in vitro and in vivo using TCR transgenic mouse models. We aim at identifying optimal miR candidates that could be therapeutically targeted to influence the differentiation of a Naive CD4+ T cells into SCM or CM D4+ T cells capable of targeting tumor cells.

P.E4.01.16 T cell cooperativity shapes antigen-specific immune responses

S. Zerneki1, J. Braun1, J. Behman2, A. Gavrilov1, N. Byersdorf1, P. Alchele2, T. Lämmermann2, T. Schumacher2, J. Rohr1

1Center for Chronic Immunodeficiency, Freiburg, Germany, 2Leiden Academic Centre for Drug Research, Leiden, Netherlands.

Upon activation T lymphocytes rapidly aggregate and form cell communities both in vitro and in vivo in an LFA1-ICAM1 dependent manner. The close proximity of cells within clusters promotes internalization of cell surface transmembrane messengers and/or direct cell interaction. We hypothesized that within clusters activated T cells mutually regulate their behaviors and subsequent differentiation akin to quorum-regulation of bacteria. In order to identify molecules involved in such crosstalk, we have developed a bioinformatics-framework allowing us to identify receptor-ligand pair co-expressed on activated T cells. We then characterized selected interactions and revealed that within clusters cellular crosstalk sustains T cell expansion by limiting activation-induced cell death. Furthermore, we found evidence for an additional related counter-regulatory signaling circuit which limits further T cell expansion. Competition between these two signaling circuits shapes the outcome of T cell activation. In summary, our results indicate that T cell clusters serve as hubs for mutual regulation of activated T cells. Such behavioral coordination within a defined spatiotemporal framework enables the generation of robust yet flexible immune responses at the population level.

P.E4.01.17 Loxosceles’ spider phospholipases D activates the PI3K/Akt signaling pathway, and join to lipid raft present in human skin fibroblasts.

J. M. Rojas Morales1, T. Irán Sekul2, J. E. Araya3, A. Catalán3

1Universidad de Antofagasta, Antofagasta, Chile.

Introduction: Phospholipase D (PLD) family enzymes from Loxosceles spider venom, are responsible for dermonecrosis during human spider envenomation. On human skin fibroblasts, different recombinant isoforms of PLDs from L. laeta, induced IL-6, IL-8, CXCL1, and CCL2/MCP-1 production. However, the signaling pathway implicated in this process is unknown. Aim: Evaluate the signaling pathway involved in chemokines production mediated by Loxosceles PLDs on skin fibroblast. Methodology: Human skin fibroblasts HFF-1 cultures were incubated with recombinant PLDs from L. laeta (rPLD1 and rPLD2), and the mutant isoforms of rPLD1 (rPLD1-D259G and rPLD1-D256S), and the Akt activation were evaluated by western blot, using an anti-phospho-Akt/PI3Kα (Ser473) monoclonal antibody. Additionally, PI3K participation was determined by Western Blot of phosphorylated Akt in presence or absence of PLDs plus wortmannin. The PLDs-lipid raft binding was evaluated by immunofluorescence using kit Vybrant® Lipid Raft labeling kit (Molecular Probes Inc.). The phospholipase D activity of PLDs was determined by Amplex Red Sphingomyelinase Assay.

Results: The recombinant PLDs from L. laeta activate Akt between 5-15 min in fibroblast cultures. This effect was decreased in cultures treated with rPLDs and wortmannin. Also, the substrate deficient rPLD1 mutants, showed lower activation of PI3K/Akt pathway compared to native Akt. Also, PLD binding to lipid rafts from fibroblasts plasmatic membrane seem to be involved in PI3K/Akt pathway activation. Conclusion: PI3K/Akt signaling pathway is activated by rPLDs, involved binding to lipid rafts present in the plasma membrane of skin fibroblasts, suggesting a role of the latters in the chemokines expression during Loxosceles envenomation.

P.E4.01.18 Lymphocyte-specific tyrosine-protein kinase Lck homo-dimers in the complex control of T-cell receptor signaling

P. Schatzmiller1, F. Baumgart1, P. Eckerstorfer1, S. Krapfl2, G. Schatz1, H. Stolzinger2

1Medical University of Vienna, Vienna, Austria, 2Institute of Pathophysiology and Allergyresearch, Medical University, Vienna, Austria.

Engagement of the T-cell antigen receptor (TCR) initiates a signaling cascade resulting in T-cell activation, proliferation and differentiation. Intracellular lymphocyte-specific kinase (Lck) plays a pivotal role in this process, transducing initial TCR/CD3 stimulation into tyrosine phosphorylation, calcium fluxing, synapse formation, and altered gene expression. Lck activity is regulated on multiple intercalated levels, including its subcellular localization (by transporters), 2D nano-domain distribution within the plasma membrane, and its phosphorylation status that is directly linked to its enzymatic activity. A potential mechanism of Lck regulation not investigated so far is its homodimerization. Noteworthy, ligand-induced homo-association followed by trans-activatory auto-phosphorylation is an established principle for transmembrane receptor tyrosine kinases.

Employing a super-resolution imaging technique - Thinning Out Clusters while Conserving the Stoichiometry of Labeling - we identified a significant amount of Lck dimers in living T-cells. Furthermore, homo-association of membrane-anchored Lck was confirmed by co-immunoprecipitation. To investigate the role of Lck homo-dimers in T-cell signaling, we established an inducible Lck-dimerization system in human Jurkat T-cells after CRISPR/Cas9 knock-out of endogenous Lck. Controlled and specific dimerization of Lck by a membrane-permeable X-linking agent significantly altered its phospho-status and enzymatic activity in a straigtforward, modularly evolving early as late TCR signaling events. In conclusion, homo-dimerization of Lck represents a novel regulatory mechanism controlling Lck kinase activity and thus stimulatory thresholds for T-cell activation.

Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands 525
Abstracts of the 5th European Congress of Immunology - ECI 2018 - Amsterdam, The Netherlands

526

POSTER PRESENTATIONS

P.E4.01.20
HyperIgM syndrome caused by defect in CD40
N. Khochot1, S. Khalisalu1, L. Smoot2, R. Yah1, S. Ottman1, F. Benhassine1, N. Attal1;
1Institut Pasteur d’Algerie, Algiers, Algeria, 2Department of Pediatrics, EPH Biologhine, Algiers, Algeria.

Introduction: Hyper IgM Syndrome (HIGM) is a rare primary immunodeficiency with defects in immunoglobulin (Ig) class switch recombination. So, the affected patients have low levels of IgG and IgA and normal or elevated IgM, resulting in high susceptibility to infections. This defect can be caused by alteration in T-B (CD40-CD40L) interaction or by intrinsic B cell defects. Germinal centers cannot be formed. The most common form is X-linked inherited and is due to mutations in CD40 ligand (CD40L) gene. In this study we report the first case of Algerian patient with HIGM caused by defect in CD40.

Materials/Methods: A male patient aged of 10 months referred us for exploration. Immunological techniques used are: measurement of IgG, IgA and IgM by nephelometry. T, B, NK immunophenotyping, evaluation of CD40, CD40 L expression and numeration of CD27 + memory B cells by flow cytometry.

Results: The patient had a medical history of pneumonia, recurrent otitis until 8 months. Studying his medical documents revealed an episode of Pneumocystis jiroveci pneumonia. He also suffered from persistent oral candidiasis. He has low levels of IgG, IgA, normal levels of IgM and normal expression of CD40L. Furthermore, he has decreased memory B cells and lack of CD40 expression on monocytes and B lymphocytes.

Conclusion: The clinical phenotype of our patient is that of a combined immunodeficiency similar to patients with CD40 defect. Lack of CD40 enables us to confirm the diagnosis of hyper IgM syndrome and consequently its autosomal recessive inheritance.

P.E4.01.21
Toll-like receptor activation by lipid nanoparticle delivered messenger RNA
S. Bates1, M. Ingenbleek2, M. Bérand3, N. Gay4, C. Betts1;
1Pathology, Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom, 2New Modalities, Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom, 3Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom.

Lipid nanoparticles (LNPs) are the most developed delivery formulations for messenger RNA (mRNA) therapy. They aid cellular delivery of mRNA and prevent extracellular degradation with proven efficacy, both in vivo and in vitro. A key safety issue is the activation of an innate immune response. Understanding the cellular mechanisms and activated inflammatory pathways are key to improving mRNA/LNP design, and hence their therapeutic potential. Here we investigate if mRNA/LNP delivery activates pattern recognition receptors such as Toll-like receptors (TLRs). Primary human dermal fibroblasts were treated with different TLR7 and TLR9 agonists in different concentrations. Intracellular signaling pathways were measured at 24 hours. The effect on proinflammatory cytokines and exogenous mRNA protein expression (luciferase) were measured. Of eight cytokines assessed, only G-CSF, IP-10, IL-8 and IL-6 were detectable. Whilst TLR4 inhibition did not impact cytokine release induced by this LNP, inhibition of downstream proteins such as IRAK4 and MyD88 led to significant drops in cytokine levels. Additionally, inhibition of endosomal formation by dynamin inhibition resulted in the largest drop in all detected cytokines, suggesting that endosomal uptake of LNP/mRNA is required to induce inflammation. This initial data supports a novel TLR signaling in mRNA/LNP activation of fibroblasts and work is ongoing to further define the mechanisms of activation and the specific TLR receptors involved.

P.E4.01.22
The ERBB-STAT3 Axis Drives Tasmanian Devil Facial Tumor Disease
1CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, 2Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria, 3University of Toronto, Toronto, Canada, 4Department of Biological Science, University of Southampton, Southampton, United Kingdom, 5Transmissible Cancer Group, Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom.

The marsupial Tasmanian devil (Sarcophilus harrisii) faces extinction due to transmissible devil facial tumor disease (DFTD). To unveil the molecular underpinnings of DFTD, we designed an approach that combines sensitivity to drugs with an integrated systems-biology characterization. Sensitivity to inhibitors of the ERBB family of receptor tyrosine kinases correlated with their overexpression, suggesting a causative link. Proteomic and DNA methylation analyses revealed tumor-specific signatures linked to oncogenic signaling hubs designed an approach that combines sensitivity to drugs with an integrated systems-biology characterization. Sensitivity to inhibitors of the ERBB family of receptor tyrosine kinases correlated with their overexpression, suggesting a causative link. Proteomic and DNA methylation analyses revealed tumor-specific signatures linked to oncogenic signaling hubs.

P.E4.01.23
The Differentiation of Human Amniotic Fluid Mesenchymal Stem Cells into Cardiomyocyte-Liked Cells
S. Aungshuawan1, R. Markmee1, P. Pothacharoen1, A. Lercher4, B. Wingelhofer1, S. Narakornsak2, T. Leowaniwatwana3;
1Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, 2Thailand Excellence Center for Tissue Engineering and Stem Cells, Department of Biochemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

The aim of this study was to investigate the efficiency of ascorbic acid (AA) on the effects on cardiogenic differentiation of human amniotic fluid mesenchymal stem cells (hAMSCs). The result of immunofluorescence and immunoenzymatic staining of the AA combined with 5-aza treatment group revealed the highest expression of cardiac specific proteins including GATA4, cTnT, Cx43 and Nkx2.5. It could be concluded that AA might be a cardiogenic inducing factor for mesenchymal stem cells and may open new insights into future biomedical applications for cardiogenic differentiation.

P.E4.01.24
Inhibitory of Rhinacanthus nasutus (L.) Kurz leaf extract on melanogenesis in B16F10 melanoma cells
B. Pratoomthai1, W. Gengnoongwong2,1, J. Naowaporn1, S. Tangtivasin1;
1Department of Basic Medical Science, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand, 2Centre Shrima, Faculty of Science, Mahidol University, Bangkok, Thailand, 3National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Pathum Thani, Thailand, 4Division of Pharmacology, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand, 5Division of Anatomy, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand.

Hyperpigmentation of the skin results from excessive melanin formation in melanocytes, and over production of melanin frequently leads to melanoma. The aim of this study was to investigate the potential of Rhinacanthus nasutus L. Kurz leaf water extract on inhibition of melanin formation or melanogenesis. R. nasutus leaf water extract was evaluated in vitro for its inhibitory effect on mushroom tyrosinase activity and cellular tyrosinase activity. B16F10 mouse melanoma cells were cultured with R. nasutus leaf extract and their tyrosinase activity and melanin content was compared with koic acid, a known tyrosinase inhibitor. Moreover, the level of expression of melanogenesis related genes and proteins were determined by quantitative RT-PCR and enzyme-linked immunosorbent assay (ELISA), respectively. The result showed that R. nasutus leaf extract had no inhibitory effect on mushroom tyrosinase activity. However, R. nasutus leaf extract significantly suppressed cellular tyrosinase activity and melanin production in B16F10 melanoma cells without any apparent cytotoxicity. Quantitative RT-PCR and ELISA revealed that R. nasutus leaf extract downregulated the expression of microphthalmia-associated transcription factor (MITF) and tyrosinase mRNAs and proteins. Taken together, the data suggest that R. nasutus leaf extract may act as an anti-melanogenic agent by inhibiting the expression of MITF and cellular tyrosinase activity. R. nasutus leaf extract may show potential as an ingredient in skin-whitening cosmetics or as a topical agent for the treatment of hyperpigmentation disorders.
Author Index
Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.
Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Presentation Number(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faye, I.</td>
<td>P.D1.03.19</td>
</tr>
<tr>
<td>Favot, L.</td>
<td>P.B2.04.18</td>
</tr>
<tr>
<td>Farriol, R.</td>
<td>WS.B5.03.06</td>
</tr>
<tr>
<td>Farinacci, M.</td>
<td>P.B4.01.16</td>
</tr>
<tr>
<td>Farina Sarasqueta, A.</td>
<td>WS.B4.01.05</td>
</tr>
<tr>
<td>Falkenburg, F.</td>
<td>P.B1.03.20, WS.B1.04.05</td>
</tr>
<tr>
<td>Falkenburg, F. H.</td>
<td>WS.E1.01.01</td>
</tr>
<tr>
<td>Faicchia, D.</td>
<td>P.C4.02.10, P.D4.05.07, P.D4.07.07, WS.B2.01.04</td>
</tr>
<tr>
<td>Fairbairn, L.</td>
<td>P.B3.04.09</td>
</tr>
<tr>
<td>Faires, B. P.</td>
<td>P.A1.01.21, P.C6.01.03</td>
</tr>
<tr>
<td>Farlie, D.</td>
<td>WS.D1.01.02</td>
</tr>
<tr>
<td>Fajtova, A.</td>
<td>P.D1.03.05, P.D1.03.12</td>
</tr>
<tr>
<td>Fakhfash, R.</td>
<td>P.A4.05.06</td>
</tr>
<tr>
<td>Fakihmi, M.</td>
<td>P.B3.04.12</td>
</tr>
<tr>
<td>Falayeveya, T.</td>
<td>WS.B4.02.17, P.B1.02.09</td>
</tr>
<tr>
<td>Falcone, M.</td>
<td>P.C1.08</td>
</tr>
<tr>
<td>Falci, T.</td>
<td>WS.A2.04.01</td>
</tr>
<tr>
<td>Falk, C. S.</td>
<td>P.A1.02.15, P.C3.02.03, P.C3.03.04, P.C3.05.09, WS.C5.01.02</td>
</tr>
<tr>
<td>Falkenburg, H.</td>
<td>WS.B1.06.05, WS.C3.02.06</td>
</tr>
<tr>
<td>Falkenburg, F. J.</td>
<td>WS.B1.06.05, WS.C5.01.02</td>
</tr>
<tr>
<td>Falkenburg, F. K.</td>
<td>WS.B1.04.05, P.B4.01.13, P.B1.05.10, P.B1.05.12, Falkenburg, J. H.</td>
</tr>
<tr>
<td>Falk Paulsen, M.</td>
<td>WS.B1.01.03</td>
</tr>
<tr>
<td>Fallon, P. G.</td>
<td>P.B4.03.13, P.C6.04.08, P.D4.03.01</td>
</tr>
<tr>
<td>Fan, Y.</td>
<td>P.B3.03.01, WS.B4.02.05</td>
</tr>
<tr>
<td>Fang, G.</td>
<td>WS.B1.07.15</td>
</tr>
<tr>
<td>Fang, Z.</td>
<td>P.B3.07.03, P.B3.07.15, P.B3.07.16</td>
</tr>
<tr>
<td>Faniello, C.</td>
<td>P.B2.07.06</td>
</tr>
<tr>
<td>Faraj, S.</td>
<td>P.B1.06.02</td>
</tr>
<tr>
<td>Fanore, C.</td>
<td>WS.B1.07.16</td>
</tr>
<tr>
<td>Fares, J.</td>
<td>P.B2.07.04</td>
</tr>
<tr>
<td>Farhat, N.</td>
<td>P.A4.06.07</td>
</tr>
<tr>
<td>Farina Sarasqueta, A.</td>
<td>WS.B4.01.05</td>
</tr>
<tr>
<td>Farina, C.</td>
<td>P.C4.02.11</td>
</tr>
<tr>
<td>Farina, F.</td>
<td>P.B2.06.02, P.C6.06.06</td>
</tr>
<tr>
<td>Farinacci, P.</td>
<td>P.B4.01.16</td>
</tr>
<tr>
<td>Farkas, N.</td>
<td>P.C6.04.03</td>
</tr>
<tr>
<td>Farmaki, E.</td>
<td>P.B2.06.15</td>
</tr>
<tr>
<td>Farambi, E.</td>
<td>P.C4.01.11</td>
</tr>
<tr>
<td>Farré, D.</td>
<td>P.D1.04.01</td>
</tr>
<tr>
<td>Farroil, C.</td>
<td>P.B2.08.03</td>
</tr>
<tr>
<td>Farrar, D.</td>
<td>WS.C3.01.04</td>
</tr>
<tr>
<td>Fassmann, A.</td>
<td>WS.B6.03.10</td>
</tr>
<tr>
<td>Fattacioli, G.</td>
<td>P.C3.04.10</td>
</tr>
<tr>
<td>Fathi, M.</td>
<td>P.A4.06.08</td>
</tr>
<tr>
<td>Fatmaoui, C.</td>
<td>P.C6.06.05</td>
</tr>
<tr>
<td>Fatoullin, C.</td>
<td>P.B2.02.19</td>
</tr>
<tr>
<td>Faurat, C.</td>
<td>P.B2.07.04</td>
</tr>
<tr>
<td>Fax, T.</td>
<td>P.A2.04.03</td>
</tr>
<tr>
<td>Favier, B.</td>
<td>P.D4.07.04</td>
</tr>
<tr>
<td>Favot, L.</td>
<td>P.B2.04.18</td>
</tr>
<tr>
<td>Faye, P.</td>
<td>P.D1.03.19</td>
</tr>
<tr>
<td>Farinacci, P.</td>
<td>P.D6.04.01, P.D6.04.09, P.D4.03.01</td>
</tr>
<tr>
<td>Even, G.</td>
<td>WS.C6.01.03</td>
</tr>
<tr>
<td>Evenroed, B.</td>
<td>WS.C6.01.03</td>
</tr>
<tr>
<td>Everts, B.</td>
<td>P.A2.02.07, P.C6.03.07, WS.A5.02.06, WS.C2.01.04</td>
</tr>
<tr>
<td>Eggeveld, A. A.</td>
<td>P.D4.05.11</td>
</tr>
<tr>
<td>Evrand, B.</td>
<td>P.B4.01.11</td>
</tr>
<tr>
<td>Evrand, M.</td>
<td>P.C1.05.03</td>
</tr>
<tr>
<td>Even, E.</td>
<td>WS.B1.02.01</td>
</tr>
<tr>
<td>Even, K.</td>
<td>P.D2.02.07</td>
</tr>
<tr>
<td>Evings, K.</td>
<td>P.B2.06.05</td>
</tr>
<tr>
<td>Eyoh, A.</td>
<td>P.A3.07.04</td>
</tr>
</tbody>
</table>

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Title</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gherardin, N. A.</td>
<td>P.B1.07.15, WS.D1.02.03</td>
<td></td>
</tr>
<tr>
<td>Ghiboub, M.</td>
<td>P.A6.02.17</td>
<td></td>
</tr>
<tr>
<td>Ghobrial, R. M.</td>
<td>P.C3.03.10</td>
<td></td>
</tr>
<tr>
<td>Ghordi, A.</td>
<td>P.B3.02.12</td>
<td></td>
</tr>
<tr>
<td>Ghoneim, H.</td>
<td>P.E4.02.18, P.B3.03.01, WS.B4.02.05</td>
<td></td>
</tr>
<tr>
<td>Ghonim, M. A.</td>
<td>P.W5.02.02</td>
<td></td>
</tr>
<tr>
<td>Ghorbani, A.</td>
<td>P.B1.08.18</td>
<td></td>
</tr>
<tr>
<td>Ghosh, A.</td>
<td>P.C1.02.13</td>
<td></td>
</tr>
<tr>
<td>Ghosh, S.</td>
<td>BS.B.01.03</td>
<td></td>
</tr>
<tr>
<td>Giacomin, P.</td>
<td>P.C2.06.03, P.D4.08.02, P.D4.09.03</td>
<td></td>
</tr>
<tr>
<td>Giamalas, P.</td>
<td>P.A3.07.14</td>
<td></td>
</tr>
<tr>
<td>Gkougkourelas, I.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilard, C.</td>
<td>P.C3.04.03, WS.C3.02.01</td>
<td></td>
</tr>
<tr>
<td>Gilardone, M.</td>
<td>P.D4.08.05</td>
<td></td>
</tr>
<tr>
<td>Gilardone, M.</td>
<td>P.C6.02.19</td>
<td></td>
</tr>
<tr>
<td>Gilardone, M.</td>
<td>P.C6.05.06</td>
<td></td>
</tr>
<tr>
<td>Gil, N.</td>
<td>P.C3.02.06, WS.C3.02.06</td>
<td></td>
</tr>
<tr>
<td>Gilbey, J.</td>
<td>P.C6.02.15</td>
<td></td>
</tr>
<tr>
<td>Gilchrist, J. P.</td>
<td>P.C1.02.17</td>
<td></td>
</tr>
<tr>
<td>Gilfeather, D.</td>
<td>P.A1.02.16</td>
<td></td>
</tr>
<tr>
<td>Gilfeather, D.</td>
<td>P.A1.02.16</td>
<td></td>
</tr>
<tr>
<td>Giller, G.</td>
<td>P.C3.02.06, P.B3.03.16</td>
<td></td>
</tr>
<tr>
<td>Giltuk, S.</td>
<td>P.D4.08.05</td>
<td></td>
</tr>
<tr>
<td>Goebel, M.</td>
<td>P.B2.04.04</td>
<td></td>
</tr>
<tr>
<td>Goebel, M.</td>
<td>P.B2.04.04</td>
<td></td>
</tr>
<tr>
<td>Gockel, S.</td>
<td>P.C3.02.06, WS.C3.02.06</td>
<td></td>
</tr>
<tr>
<td>Gockel, S.</td>
<td>P.C6.02.19</td>
<td></td>
</tr>
<tr>
<td>Goel, K.</td>
<td>P.A3.01.08</td>
<td></td>
</tr>
<tr>
<td>Goen, S.</td>
<td>P.C6.03.04, P.B6.01.08</td>
<td></td>
</tr>
<tr>
<td>Goen, S.</td>
<td>P.C6.03.04, P.B6.01.08</td>
<td></td>
</tr>
<tr>
<td>Gole, A.</td>
<td>P.A3.05.16</td>
<td></td>
</tr>
<tr>
<td>Goldmann, K.</td>
<td>P.C5.01.06</td>
<td></td>
</tr>
<tr>
<td>Goldmann, K.</td>
<td>P.C5.01.06</td>
<td></td>
</tr>
<tr>
<td>Goldstein, S.</td>
<td>P.C1.02.17</td>
<td></td>
</tr>
<tr>
<td>Golc, O.</td>
<td>P.B3.02.12</td>
<td></td>
</tr>
<tr>
<td>Golo, J.</td>
<td>P.D3.01.08</td>
<td></td>
</tr>
<tr>
<td>Golubtsov, V.</td>
<td>P.C3.06.16</td>
<td></td>
</tr>
<tr>
<td>Gombart, J. M.</td>
<td>P.B3.01.04, P.B3.01.05</td>
<td></td>
</tr>
<tr>
<td>Gomila, P.</td>
<td>P.C1.02.17, P.A1.01.05</td>
<td></td>
</tr>
<tr>
<td>Gomila, P.</td>
<td>P.C2.01.05</td>
<td></td>
</tr>
<tr>
<td>Gomila, P.</td>
<td>P.C2.01.05</td>
<td></td>
</tr>
<tr>
<td>Gomila, P.</td>
<td>P.C2.01.05</td>
<td></td>
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<tr>
<td>Gomila, P.</td>
<td>P.C2.01.05</td>
<td></td>
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<tr>
<td>Gomila, P.</td>
<td>P.C2.01.05</td>
<td></td>
</tr>
<tr>
<td>Gomila, P.</td>
<td>P.C2.01.05</td>
<td></td>
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<tr>
<td>Gomila, P.</td>
<td>P.C2.01.05</td>
<td></td>
</tr>
<tr>
<td>Gomila, P.</td>
<td>P.C2.01.05</td>
<td></td>
</tr>
<tr>
<td>Gomila, P.</td>
<td>P.C2.01.05</td>
<td></td>
</tr>
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Gutowski-Osiak, D.: P.C1.04.06
Guinec, G.: P.A2.05.02
Guizek, A.: P.D1.04.05
Guzmán, C. A.: P.A3.05.17
 Györgyosi, Á.: P.B2.04.02
Gyurvesi, L.: P.C3.09.12
H\textit{aabheth, O. A.: W.S.04.02.05}
 Haack, S.: W.S.C1.01.02
 Haake, E.: P.C1.05.09
 Haff, A.: P.C8.07.16, P.C8.06.16, P.C6.02.12
Hagman, H.: P.D7.04.16, P.D7.04.17
Habans, J. B.: B.P1.06.11, B.P4.01.02
Haastra, R.: W.S.03.04.03
Haas, R.: P.A1.02.01
Habenberger, P.: P.C5.03.10
Habenicht, A.: P.C6.05.02
Haddadin, H.: P.A2.03.12
Hajberry, M.: P.B4.05.05
Hajec, C.: P.C1.02.03
Hahne, A.: P.D3.01.01
Hagen, M.: P.C1.03.08
Hagemann, P.: P.C1.11.07
Hagemeyer, N.: P.A1.01.06
Hagen, M.: P.A3.07.06
Hägi, D.: P.C2.05.03
Hake, J. C.: P.D1.05.10
Halk, M.: P.B3.02.02
Hammerfors, D.: P.C2.03.11
Hammer, Q.: P.D1.02.12
Hämmerl, L.: P.D1.01.18, W.S.C1.01.01
Hammers, C.: P.E3.04.16
Hammering, P.: P.A1.02.18, P.A4.04.01, P.C3.02.05
Hamp, D.: W.S.C4.02.06
Ham, M.: P.B4.02.08
Hamon, Y.: P.C6.06.05
Hammouri, S.: W.S.D3.01.06
Hams, E.: P.B3.04.13
Hamke, M.: P.C2.07.09
Ham, H.: B.S.D1.03.03, P.D4.01.14
Hankel, N.: W.S.A2.05.02
Harisch, H.: P.C2.04.07
Haeberle, S.: W.S.C4.01.03
Haeckel, P.: P.A3.01.13
Gunes, N.: P.A2.01.16
Gunes, D.: P.C2.05.12
Gülich, A. F.: P.C6.04.06
Gul, K.: P.B2.03.13
Guelf, J.: P.C2.04.11
Guitart, C.: P.C1.02.03
Guerrero, P.: P.A4.01.02
Garcia, E.: P.A4.01.19
Garcia, E.: P.C3.02.09
Gustavsson, B.: P.B3.04.01
Gülich, A.: P.C6.03.05
Gülich, A.: P.C6.02.14
Gulch, K.: P.A5.06.08
Guenn, M. C.: P.C4.01.03
Gulev, E.: P.D1.03.09
Güler, C.: P.C2.04.07
Guerrera, G.: P.D1.02.18, W.S.E1.03.03
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Guerrero, J.: P.A1.01.02
Gülich, A.: P.C6.02.05
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
Gusel, G.: P.A5.03.06
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Gusel, G.: P.A5.03.06
Gülich, J.: P.C2.04.07
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